Influence of reproductive structures on the morphology and physiology of *Pinus contorta* trees.

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ABSTRACT

The aim of this study was to determine the influence of sexual structures on the growth of Pinus contorta (Dougl.) trees. It is important to know if male and female cones reduce the growth of trees, because it is probable that seed production techniques currently used, will result in an increased level of cone production in the forests of the future.

This study initially examined the influence of male and female cones on the carbon production and allocation in individual branches of field grown trees. These studies showed that, compared with closely associated vegetative branches: (i) when the cones were fully developed reproductive branches had more dry weight, (ii) female cones reduced neither number of laterals nor the length of the terminal or lateral shoots on the branches which bore them, (iii) male cones reduced both the number of laterals and number of needles on the shoot which bore them, (iv) needles on male cone-bearing branches had significantly higher photosynthetic rates compared with needles on vegetative branches in the autumn, and needles on female cone-bearing branches generally also fixed more CO_2 per quanta. In addition both male and female cones refixed a significant proportion of respired CO_2 when illuminated.

Using a tested mathematical model 'MAESTRO' it was possible to estimate the influence of male and female cones on the assimilate production of a target tree within a forest stand. It was estimated that the reduction in needle complement associated with male cones would result in a slight reduction in net daily CO_2 assimilated compared with vegetative trees on sunny days in autumn. But would lead to a higher daily assimilation on cloudy days because of the associated reduction in respiration. In addition it was estimated that the refixation of respired CO_2 would result in a CO_2 efflux by female cones equivalent to only a small proportion of the CO_2 assimilated on bright sunny days. But on cloudy days, when refixation was low, female cone respiration represented a significant loss of carbon from the tree.

A potted plant system was successfully devised to allow a complete carbon budget for reproductive and vegetative plants to be

calculated. Unfortunately insufficient plants were produced during this study, probably because of environmental and pathological rather than physiological factors. This idea is therefore considered important and justifies further study.

As the production of reproductive structures did not appear to reduce the vegetative growth of trees, it was suggested that trees, through natural selection, have developed mechanisms to ensure maximum vegetative and reproductive growth simultaneously.

DECLARATION

I hereby declare that this thesis has been composed by me and that the work recorded is my own, except where acknowledged to the contrary.

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CHAPTER 1

INTRODUCTION AND AIMS

1.1 GENERAL INTRODUCTION

As the world's need for timber increases large areas of virgin conifer and tropical forests are harvested each year. These forests are a limited resource and need to be replaced for the future. In order to improve productivity of future forests 'plus' trees are commonly selected and incorporated into seed orchards. There are many techniques used by tree breeders and seed producers to enhance the cone crop of conifers (see reviews by Puritch 1972, Longman 1985, Owens & Blake 1985, Pharis *et al.* 1987). Many of the techniques used give variable results which has been attributed, at least in part, to genetic variation in the material tested. Generally the genotypes which naturally produce a good seed crop respond best to treatment. A number of studies show genetic variation in the quantity of seed crop a tree or clone naturally produces (Nienstaedt & Jeffers 1970, Andersson & Hattemer 1978, Griffin 1982, Nikkanen & Velling 1987) indicating that cone production is a heritable trait (Nienstaedt 1985). Breeding from floriferous individuals may, therefore, lead to increased cone production in future forests.

It has been asserted many times, however, that tree growth is adversely affected by cone production (Fielding 1960, Eis *et al.* 1965, Tappeiner 1969, Smith & Stanley 1969), although there is some controversy in the literature because tree growth has also been found to be positively associated with the presence of female cones (Gerhold 1966, Chalupka & Giertych 1975, Simpson & Powell 1981, Nienstaedt 1985). It was the aim of this study to determine if the development of reproductive structures on *Pinus contorta* Douglas (lodgepole pine) trees is detrimental to vegetative growth. In order to achieve this objective the literature on the subject has been reviewed and the morphology and physiology of cone-bearing branches and plants has been compared with vegetative branches and plants. The findings from these studies have been used to determine the

influence of male and female cones on forest trees by altering the appropriate morphological and physiological parameters in a simulation model of a forest stand.

1.2 DEFINITION OF TERMS

Cone A strobilus, an assemblage of sporophylls bearing sporangia arranged in a cone-shaped structure.

Female bud A bud with differentiated female cone (see Figure 1.1a).

Female cone A strobilus containing megasporangia which develop into seeds.

Lateral shoot The branch situated at the side of the main branch (see Figure 1.2b).

Male bud Bud with differentiated male cones (see Figure 1.2a).

Male cone A strobilus containing microspores often termed pollen.

Monocyclic One cycle of growth, sometimes termed uninodal (i.e. a single branch whorl per year)

n female cone Female cone in year of initiation (i.e. when still enclosed in the bud see Figure 1.1a)

n1 female cone One-year-old female cone (i.e. year of female cone pollination see Figure 1.1b)

n2 female cone Two-year-old cone (i.e. year of female cone fertilisation and seed shed. see Figure 1.1c & d)

Parent branch The previous year's main axis growth (see Figure 1.2).

Polycyclic More than one cycle of growth, sometimes termed multinodal (i.e. more than one branch whorl per year).

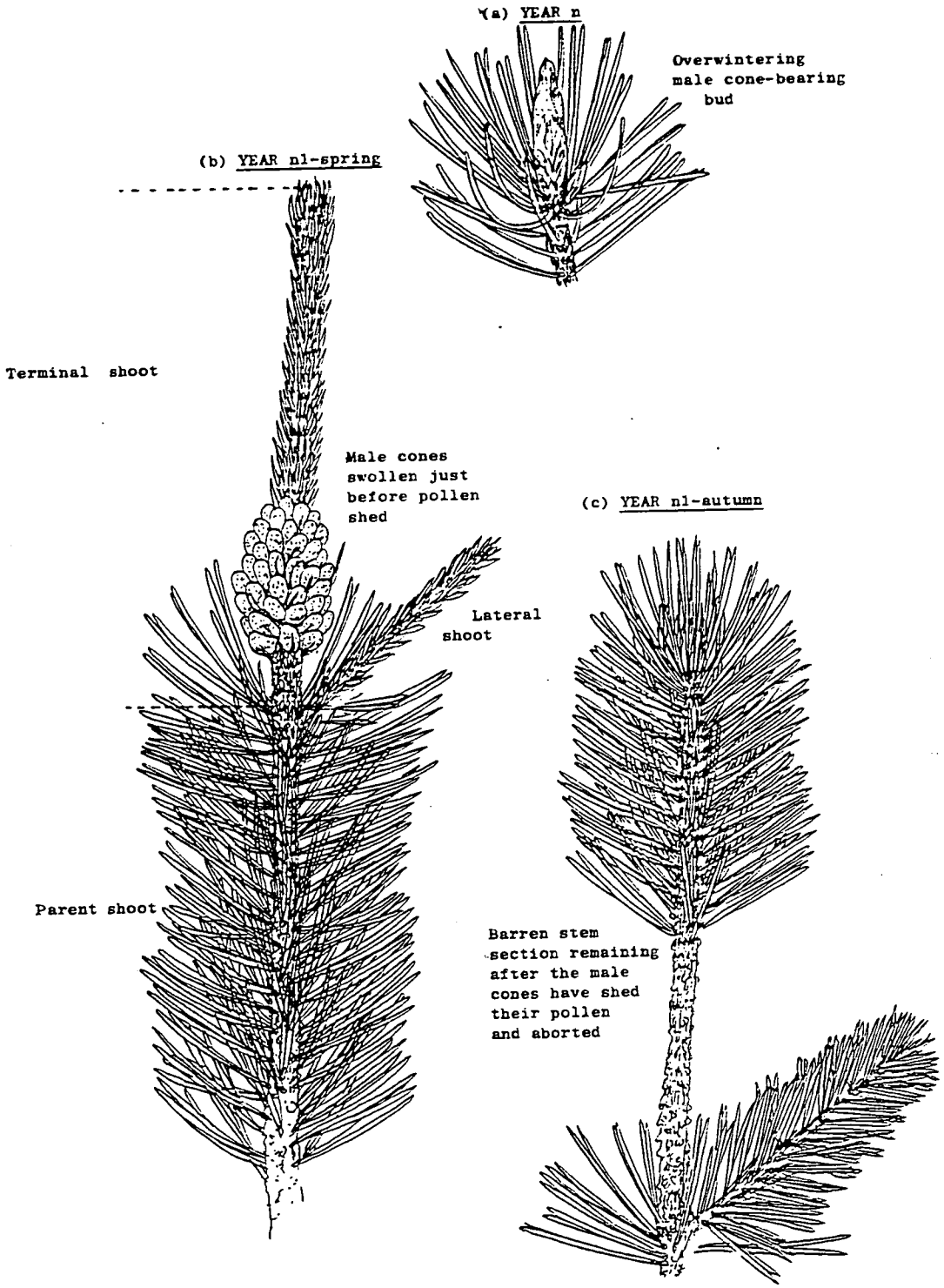


Figure 1.1 Growth and development of shoots and male cones of P. contorta.

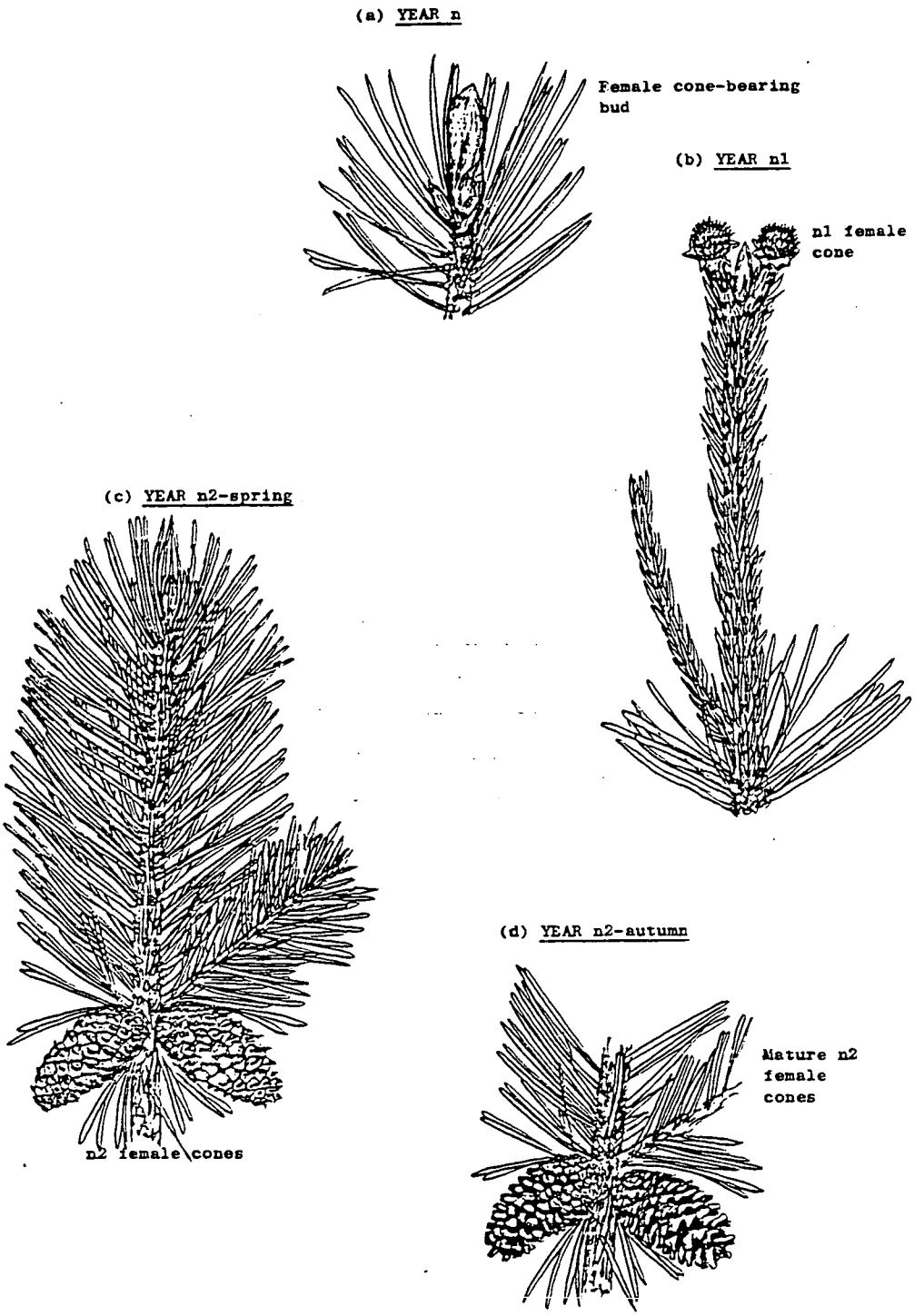


Figure 1.2 Stages in the development of female cones of P. contorta

Sterile cataphyll Bud scales without differentiated primordia.

Terminal shoot The branch situated at the tip (see Figure 1.2b).

Vegetative bud Bud with only needle and branch primordia.

1.3 INFLUENCE OF REPRODUCTIVE STRUCTURES ON GROWTH OF CONIFERS

Most conifers are wind pollinated and therefore produce large quantities of pollen. Fielding (1960) estimated that Pinus radiata (Monterey pine) produces an average annual dry-weight of between 100 - 180 kg ha^{-1} of pollen and pollen-bearing material over a rotation of 40 years. Madgwick (1985) reported that the weight of female cones varied from 1 to 9 tonnes ha^{-1} in a stand over 13-years-old. However this is low compared to the weight of the total stem wood. Madgwick et al. (1977) sampled 5 - 7 whole trees in each of 9 plots ranging from 2-22 years old, and found that the weight of female cones ranged from 0.08 tonnes ha^{-1} in the 8-year-old plot to 5.02 tonnes ha^{-1} in the 17-year-old plot but this was equivalent to only 0.3% and 2.3% of the total weight of stem wood. Other workers have estimated that cones account for 0.6% to 3% of the total above ground biomass (see Pulkkinen et al. 1989). The relatively low proportion of cone dry weight to total stem dry weight reflects, at least in part, the fact that cones occur only after the tree already has a substantial stem weight.

It is, however, the relationship between annual production of cones and stem dry weight that is more relevant. A reduction in the annual increment of stem wood caused by cone production has been reported in many species with estimates varying from 12% to 40% (see Kozlowski 1971a, Koppel et al. 1987). Eis et al. (1965) found that abundant cone production had a depressing effect on the annual ring width only during the year when cones were present on the tree, which they considered was year 1 in the life cycle of Pseudotsuga menziesii (Douglas-fir) and Abies grandis (grand fir) cones and year 2 in the life cycle of female cones of Pinus monticola (western white pine). They interpreted this to show that only current photosynthate was used in cone production.

Tappeiner (1969) also found a reduction in the annual ring width of Pseudotsuga menziesii associated with cone production. However, he suggested that the consistently narrower xylem rings on the cone-bearing trees may be explained by their larger diameters. Chalupka et al. (1975) also found a reduction (0.25-0.50 mm) in radial increment of Picea abies (Norway spruce) for years with the heaviest seed crops, but based on the stump value of the wood concluded that the reduction was not sufficient to make any action against the cone crop economically justified. They went on to show a negative correlation between cone crop and specific gravity of wood, which they suggested was an effect on the weight of individual tracheids (Chalupka et al. 1977).

Determination of the reduction in the annual radial increment of trees caused by cone production is complex because the increment of a stem is influenced by the weather and the aging of the stem as well as by the cone crop (Chalupka et. al. 1975). In addition comparative studies between cone-bearing and vegetative trees are often hindered by genetic differences and the age of the trees. For example Morris (1951) selected his non-flowering groups of trees from a much younger stand than the flowering groups. Consequently it is not clear from the literature that cone production per se causes a reduction in annual radial increment.

It has been found that generally the most vigorous dominant trees within a stand produce cones. Morris (1951) sampled 240 trees of Abies balsamea (balsam fir) in a 50-year-old stand and showed that the following percentage of trees were producing flowers: dominant, 83%; codominant, 59%; intermediates, 6%; suppressed, 0%. Other workers have also found positive relationships between number of female cones and tree height (Gerhold 1966, Simpson & Powell 1981). In addition cone production on a branch is positively correlated with branch diameter (Fielding 1960, Nikkanen & Velling 1987) and branch length (Varnell 1976). Teich (1975) found that precocious Picea glauca (white spruce) trees had an inherent ability to grow rapidly in the absence of cone production. However, his data suggest that after trees begin to produce cones, growth is slightly reduced ($p = 0.0625$). Nienstaedt (1985) also found a significant

correlation between the early production of male and female cones of Picea glauca and the fastest growing progeny. He appreciated that growth may suffer, if early flowering trees continue to produce heavy seed crops, as found by Teich (1975). However, he found an increasing correlation between female cone production and height from 8 to 15 years of age and this indicates that there was no reduction in growth associated with female cones between 8 and 15 years. This discrepancy between the early production of cones and a possible reduction in vegetative growth may result from a difference in the nutrition of the plots. Early vigorous growth of trees may result in local nutrient deficiencies developing, Nienstaedt considered that his plots were in near optimum growing conditions which may explain why he found no reduction in growth after cone production commenced.

Although cone-bearing trees grow faster than vegetative trees this may lead to a problem in some species with persistent stem-borne cones, such as Pinus radiata. Stem cones which remain in place for many years after maturity are forced radially outwards from the pith by the expanding stem. They leave a duct, usually resin filled and about 10 mm or more in diameter in the wake of the trailing cone peduncle. Occasionally, on fast growing trees, cones may become imbedded within the expanding bole (Fielding 1960, Lavery 1986).

Cones not only influence the dry weight of the component parts of a tree they also contain significant amounts of nutrients (Madgwick et al. 1977). Stewart et al. (1981) estimated that for ten sample trees in a 15-year-old stand of Pinus radiata, female cones contained the following nutrients as a percentage cone biomass: nitrogen 11%, phosphorus 15%, potassium 4%, calcium 1%, magnesium 7%, aluminium 5%, iron 9%, manganese 2%, and zinc 13%. The nutrient content of the cones was always less than that of the living foliage. Das & Ramakrishnan (1987) found for 3 to 22-year-old trees of the tropical pine, Pinus kesiya, that the concentration of nitrogen, phosphorus, potassium, magnesium and calcium decreased in the following order: needles> live branches> dead branches> boles> cones. Ovington (1963), however, estimated from litter trap collection data that male cones of Pinus strobus (Weymouth pine)

which had already shed their pollen contained higher concentrations of nitrogen, potassium and phosphorus than needles. The difference between the living material and the litter data suggest that nutrients contained in cones are retranslocated when the cones senesce (see Kozlowski 1971b) less efficiently than the retranslocation of nutrients from needles before they are abscissed (Katainen & Valtonen 1985).

1.4 MOVEMENT OF ASSIMILATES AND CO_2 EXCHANGE IN CONIFERS

Temperate conifers have a distinct seasonal growth pattern. Cells do not divide or extend during the winter when daylength is short and temperatures relatively low. However, it is not only environmental constraints which limit growth. Physiological mechanisms like dormancy appear to operate to prevent growth during the winter (Wareing 1951). In the spring when environmental conditions are more favourable, root and shoot extension occurs. Although free growth may occur in young seedlings (Thompson 1976), shoot growth of conifers is generally predetermined. Primordia are initiated during the spring and summer of one year and they elongate the following year. Both environmental and genetic factors influence the number of primordia initiated and their subsequent elongation (see review by Cannell et al. 1976).

Many workers have investigated the seasonal pattern of assimilate production and allocation in trees. A variety of techniques have been employed which have all led to a fairly consistent pattern. The techniques used include: (i) incorporation of ^{14}C (Shiroya et al. 1966, Ursino et al. 1968, Gordon & Larson 1968, Dickmann & Kozlowski 1970a, Schier 1970, Ericsson 1978); (ii) CO_2 exchange (Freeland 1952, Clark 1961, Loach & Little 1973, Fry & Phillips 1977, Drew & Ledig 1981, Troeng & Linder 1982a,b, Beadle et. al. 1985); (iii) carbohydrate analysis (Krueger 1967, Clausen & Kozlowski 1967, Little 1970ab, Olofinboba & Kozlowski 1973, Ericsson 1980, Deans & Ford 1986); (iv) defoliation and girdling (Kozlowski & Winget 1964, Ebell 1971, Ericsson 1980); and (v) mathematical modelling (Reed et. al. 1976, Chung & Barnes 1980, Linder & Lohammar 1981). The studies all show a seasonal pattern which has

been reviewed by several workers (Kozlowski & Keller 1966, Fayle 1968, Ericsson 1980, Jarvis & Sandford 1986, Oquist & Martin 1986).

In general, coniferous tree show a seasonal pattern of growth starting in the spring with root growth which usually precedes bud break. Photoassimilates previously stored in the shoots are primarily utilised in the spring. As the buds swell and break, root activity slows, and both stored and current photoassimilates are transferred into the growing shoot. Cambial growth is renewed at about the same time as bud break. Shoot extension terminates first, while cambial and root growth terminates later in the season.

Nutrients apparently follow a similar pattern to photoassimilates (see reviews by Taylor 1967, Mooney 1972). Krueger (1967) showed in seedlings of Pseudotsuga menziesii that nitrogen, phosphorus, and carbohydrates move from old to new shoots at bud break. Katainen & Valtonen (1985) examined the annual nutrient dynamics of three stands of Pinus sylvestris (Scots pine) and also showed that nutrients were mobilised for growth. They found that elements mobile via the phloem such as nitrogen, phosphorus and potassium had high retranslocation rates while calcium was almost immobile and accumulated in the needles during their lifetime.

There follows a brief summary of the seasonal growth and CO_2 exchange of the shoot, roots, stem and cones.

1.4.1 SHOOT AND NEEDLES

It appears to be a general rule of angiosperms that new leaves are net importers of photoassimilates until they reach approximately 50% of their final size (Mooney 1972, Weaver & Johnson 1985). The current year's shoot of conifers appear to follow a similar pattern (see Ericsson 1980). The majority of the carbohydrates for the initial growth of the current year's shoot are translocated from the previous year's needles (n2), although assimilates may be supplied from older needles (n3) if the n2 needles are removed (Ericsson 1978). In addition, Kozlowski & Winget (1964) estimated that

branches, main stem and roots contributed 5.7%, 4.9% and 1.8%, respectively, of the final dry weight of the current shoot. Photoassimilates produced by the older needles appear to be translocated to the stem and roots during the period of shoot extension (Ericsson 1978) with both the n1 and n2 needles also contributing after shoot extension has ceased.

The CO_2 exchange rates of needles have been extensively measured and the data summarised in a number of reviews (Larcher 1969, Schaedle 1975, Jarvis & Leverenz 1983, Jarvis & Sandford 1986). It is generally considered that the photosynthetic capacity of n1 needles is higher than that of n2 needles as soon as the foliage has reached maturity and thereafter the capacity decreases with age. (Jarvis & Sandford 1986). In Picea sitchensis (Sitka spruce) needles, for example, the maximum assimilation rate three years after emergence was reduced from 8.8 to 2.7 μmol m^{-2} s^{-1} (Ludlow and Jarvis 1971). This reduction in maximum assimilation rate was associated with a reduction in both stomatal and mesophyll conductances. Jarvis and Sandford (1986) suggest that the decrease in stomatal conductance in older needles may be caused by a reduction in the permeability of the matrix of wax rods in the stomatal antechamber, resulting from the accumulation of pollutants, spores and micro-organisms.

In addition to variation in the CO_2 exchange of needles attributed to their age there is also distinct spatial variation within a tree crown. Needles at the top of a canopy are physiologically and anatomically conditioned to high quantum flux densities compared with the needles in the lower crown (Leverenz and Jarvis 1979). Both the quantity (total quantum flux density) and quality (ratio of the wavelengths absorbed by phytochrome, ς) change with distance into a canopy. Jarvis & Sandford (1986) concluded that it was not clear to what extent differences in the assimilation rate with depth into the canopy were caused by vertical differences in ς or in quantum flux density.

There is also seasonal variation in the CO_2 exchange rates of needles. In mild climates there is little variation in the photosynthetic capacity during the year (Fry & Phillips 1977) and

the photosynthetic rate is largely related to environmental variables, primarily quantum flux density, vapour pressure deficit and temperature. However, in more extreme climates, there is a pronounced seasonal variation in photosynthetic capacity (Kozlowski & Keller 1966, Linder & Troeng 1980). During the autumn the photosynthetic capacity of needles falls and remains low until dehardening occurs in the spring (see review by Oquist & Martin 1986). In general, net photosynthesis ceases completely when water in the needles freezes extracellularly and restricts CO_2 diffusion. In addition freezing temperatures induce dehydration of the mesophyll cells, and excess light that is absorbed by chlorophyll can cause a substantial chlorophyll bleaching particularly at high elevations. Respiration is also inhibited by freezing temperatures but its activity resumes directly after thawing during the winter. This can result in conifers in the temperate region showing a negative daily carbon balance during winter (Troeng & Linder 1982a) although the carbon balance is usually positive every month (Bradbury and Malcolm 1978).

1.4.2. ROOTS

There is a general trend for a high proportion of a assimilates to be translocated to the roots early in the season before shoot growth, (Shiroya et al. 1966, Loach & Little 1973), and during the autumn after shoot extension has ceased (Ursino et al. 1968, Deans & Ford 1986). Fine roots appear to be formed continuously and then die during the growing season in times of stress. Fogel (1983) quotes references for between 30% - 86% turnover of fine roots annually. This represents a large component of the carbon balance in the tree. It has been estimated that, although constituting less than 5% of the total tree biomass, fine roots often account for a major portion of annual stand production (see review by Santantonio 1989). He quotes estimates of the proportion of total net annual primary production which was allocated to fine roots ranging from 5% in young Pinus elliottii stands to 68% in a mature Abies amabilis stand. Generally a larger amount or higher proportion of total net primary production was allocated to fine roots in stands on poor sites and as stands matured. Roots are however notoriously difficult

to study quantitatively. Typically, effects of soil water, nutrient status, stand composition, stage of stand development have not been controlled experimentally, but have been confounded within the site condition. Mycorrhizas are another complication: they may represent as much as 40% of the dry weight of absorbing rootlets in a spruce forest and it has been estimated that as much as two thirds of the net photosynthesis may be lost to mycorrhizas (see review by Mooney 1972). Mycorrhizas also complicate the interpretation of CO_2 exchange rate of roots and shoots. Reid et. al. (1983) found that mycorrhiza formation resulted both in increased shoot photosynthetic rates and root respiration rates in Pinus contorta and Pinus taeda seedlings. They concluded that increased photosynthetic rates were probably the result of improved nutrient status of the plants, while increased root respiration rates were a direct effect of mycorrhizal infection.

1.4.3 STEM

In the spring the initiation of cambial activity starts more or less at the same time in both the tree crown and at the base of the stem (Zajaczkowski 1973). The increase in stem cross-sectional area was found by Deans & Ford (1986) to be depressed by elongation of the current year's shoot and to increase after shoot extension had ceased. They suggested that when shoot and needle elongation were proceeding rapidly, substrate availability was insufficient to support stem growth. Gordon & Larson (1968) found that the production of xylem tracheids normally associated with latewood was physiologically correlated with maturation of the current season's needles. They suggested that the cambium, being a diffuse meristem, was not a concentrated sink for assimilates and could not compete effectively with the mass meristem of the developing shoot early in the growing season.

Linder & Troeng (1980) found a pronounced seasonal trend in stem respiration of Pinus sylvestris. From late autumn until the start of the growing season, the respiration rate was low, but as soon as stem growth started the respiration rate increased. Comparison of stem growth and the pattern of respiration showed that the maximum

rate of diameter increase occurred one month earlier than the peak in respiration rate. They suggested that this was the result of secondary and tertiary wall-thickening which takes place after increase in stem volume. The stems of conifers, along with many other trees, have a reduced respiration rate when illuminated because of reassimilation (see reviews by Mooney 1972, Schaedle 1975). The ability of a Pinus sylvestris stem to refix carbon dioxide released by respiration was reduced down the stem with increasing age of the stem section (Linder & Troeng 1980), possibly because the thicker bark at the base of the stem prevents light penetration (see Schaedle 1975). Branches also have a reduced rate of respiration when illuminated. Benecke (1985) estimated that branches of Pinus radiata had a mean annual CO_2 refixation of 23%.

1.4.4 CONES

In most genera of gymnosperms (e.g. Abies, Larix, Picea, Pseudotsuga) the cones or strobili ripen during the first season. However, pines of the temperate zone usually require 15 to 16 months from pollination to ripening (see review by Kozlowski 1971b). The male and female cones of Pinus have separate periods of maximum growth. Male cones slowly increase in size following initiation, stop growing during the winter (Couper 1987) and rapidly increase in size and weight before they shed their pollen in the spring. Female cones, in contrast, increase in dry weight steadily following pollination and stop growing in the autumn when they are approximately one third the length of mature cones. During the following spring they rapidly increase in length, diameter and dry weight. Female cones cease to grow in the summer, about the same time as shoot extension. Dickmann & Kozlowski (1969) suggested that the resumption of pollen-tube growth and fertilization of the ovule may act as trigger mechanisms which initiate rapid mobilization of metabolites which are responsible for the growth surge exhibited by n2 cones. Marked diurnal fluctuations in cone diameter have been noted after overall growth of the cone has been completed. Similar patterns have been found in other fruits (see review by Kozlowski 1971b) and are thought to be the result of leaf transpiration

resulting in a loss of water from the fruit during the day and a subsequent rehydration of the fruit during the night.

Reproductive structures of many plants are strong sinks for assimilates (see review by Weaver & Johnson 1985). Tracer studies have shown that n2 cones of Pinus resinosa (red pine) are a high priority sink for assimilates (Dickmann & Kozlowski 1968, 1970). The recovery of ^{14}C from the various sinks studied was in the following order n2 cones> terminal needles> lateral needles> terminal internode> lateral internode> 1-year-old-wood. They concluded that carbohydrate reserves were important early in the growing season, but as these become depleted, currently produced carbohydrates, particularly from the one-year-old needles and current needles late in the season, became the dominant carbohydrate source for cone development. Many studies (including studies on apple trees and wheat) have shown that the largest part of the carbon utilised in the development of flowers and fruit comes from the leaves nearest them (see review by Mooney 1972).

It has been found for female cones which ripen during the first season, for example cones of Pseudotsuga menziesii, that respiration rates generally increase gradually until pollination and then decline before senescence (Ching & Ching 1962). Following pollination, seeds within the cone had a higher respiration rate than the surrounding cone scales (Ching & Fang 1963). However, in Pinus sylvestris, which take longer to mature, the respiration rate was found to reach a peak during the second year of cone development, which, it was suggested, coincided with fertilisation of the female gamete (Linder & Troeng 1981). No similar measurements of respiration rates of male cones could be found in the literature. In a similar way as has been described for stems, the fruits and flowers of many species may have a reduced rate of respiration when illuminated, thereby contributing to there own carbon economy. In young green fruits, 20%-80% of the CO_2 released in dark respiration may be conserved through refixation, but the efficiency is reduced as the fruits mature (see reviews by Mooney 1972, Schaedle 1975, Blake & Lenz 1989).

1.5 CONTROL OF ASSIMILATE PRODUCTION AND ALLOCATION

Although the biochemistry of assimilate production (photosynthesis) has been known for a long time, the mechanism by which assimilate production is controlled in plants is not fully understood. Several factors influence photosynthesis and could be considered as control mechanisms:

(i) Light activation of photosynthesis. As light is a major driving variable in the process of CO_2 fixation in plants it clearly influences the rate of photosynthesis (see review by Jarvis & Sandford 1986).

(ii) Nutrient deficient inhibition of photosynthesis. Poor nutrient status of a plant can limit photosynthesis. Increased photosynthetic rates following increased nutrient supply have been attributed to increased chlorophyll production, increased stomatal and mesophyll conductance to CO_2 diffusion, increased activity of carboxylating enzymes, and increased leaf size (see Ekwebelam & Reid 1984).

(iii) CO_2 limitation of photosynthesis. In addition to the reduction in photosynthesis caused by a low ambient concentration of CO_2, the transfer of CO_2 into the photosynthesising cells may be limited by: (a) a low boundary layer conductance, and (b) a low stomatal conductance. Photosynthesis may also be limited by the activity of the carboxylase enzyme, often encapsulated in the mesophyll conductance, (see review by Jarvis & Sandford 1986). Weaver & Johnson (1985) concluded there was increasing evidence of hormonal control of photosynthesis mediated through effects on the stomatal conductance.

(iv) Feed-back inhibition of photosynthesis through end product control. Three mechanisms for end product control have been proposed (a) direct feedback control of photosynthesis by the products, (b) physical effects of starch accumulation on chloroplast or CO_2 diffusion, and (c) remote or hormonal feedback effects on photosynthetic metabolism (see review by Bidwell 1983). Neales & Incoll (1968) reviewed the hypothesis that photosynthesis was

controlled by the concentration of assimilates in leaves. They concluded that although there was undoubtedly a relationship between the photosynthesis of a leaf and the carbohydrate content there was no proof that the two were causally related. More recently Bidwell (1983) also considered this question and concluded that direct feedback control of photosynthesis by products of photosynthesis does not occur, except in a minor way through the mechanical effects of a glut of photosynthate. He noted that there were many observations of the effect of sink demand for assimilates which have led to the general concept that photosynthesis may be regulated by a sink signal possibly of hormonal nature (see also reviews by Weaver & Johnson 1985, Chalmers 1985).

Bidwell (1983) concluded that photosynthesis was probably controlled by a network of unrelated systems which responded to environmental and physiological signals.

The relationship between the source and sink activity of plant parts has received much attention because most increases in productivity, particularly in agriculture, have resulted from increasing the proportion of assimilates allocation to the harvestable parts (Pulkkinen et al. 1989). Weaver & Johnson (1985) reviewed the regulation of assimilates within plants and found the following general rules: (i) young leaves are sinks for assimilates until they are approximately 33% to 50% expanded after which time they export assimilates, initially to the apex but after new leaves have been produced the movement of assimilates becomes primarily basipetal; (ii) old leaves seldom import assimilates, thus the sink strength of tissues constantly changes during the growth of the plant; (iii) reproductive organs are usually stronger sinks for assimilates than vegetative parts; (iv) the leaves nearest a sink are the predominant source of assimilates; (iv) the strength of a sink is a measure of the sinks ability to utilise assimilates and thereby create a concentration gradient in the sink's favour; and (v) auxins, gibberellins and cytokinins can effectively promote the strength of a sink whereas inhibitors such as abscisic acid, daminozide and chormequat often decrease the ability of a sink to attract assimilates.

Weaver & Johnson (1985) concluded that the movement of photosynthetic assimilates to a sink is enhanced by a decrease in available carbohydrate in the sink tissue, thus resulting in a higher concentration gradient towards the sink. The number of sinks and the distance from the site of assimilate production to the sink, also influence mobilization of assimilates. Both the male and female cones are ideally positioned on the shoot in terms of distance from a major source of assimilates. Male cones are formed in the base of a bud which consequently means they are the 'first sink' that assimilates moving distally from the n/n1 needles would encounter (Figures 1.2). On the other hand, female cones are formed at the tip of the growing bud. But as it is two years before their period of maximum growth they are also the 'first sink' that the assimilates would encounter moving distally when the cone is rapidly increasing in size and weight (Figure 1.3). Thus, both male and female cones are morphological situated in the ideal position on the shoot, as they are the closest sink to the most productive needles on the tree during their period of maximum weight increment. Weaver & Johnson (1985) also noted that assimilate transport was probably controlled by plant growth regulators especially gibberellins and auxins. Both of these plant growth regulators are associated with the production of male and female cones, and it has been suggested that their mode of action is by assimilate attraction according to the so called 'nutrient diversion theory' (see reviews by Sachs & Hackett 1977, Dunberg & Oden 1983, Ross & Pharis 1985).

1.6 TAXONOMY AND ECOLOGY OF PINUS CONTORTA

The Pinaceae is the most important family of commercially planted conifers around the world. Pinus contorta Douglas (lodgepole pine) was chosen as a test species in this study because it is a commercially important pine which cones regularly in Scotland. It is native to western North America where it has a wide natural range, along the coast from southern California to Alaska and inland to the Rocky Mountains. Pinus contorta extends over a wider range of elevations than any other species in North America, from sea level at the Pacific to around 3400 m in the Southern Sierra

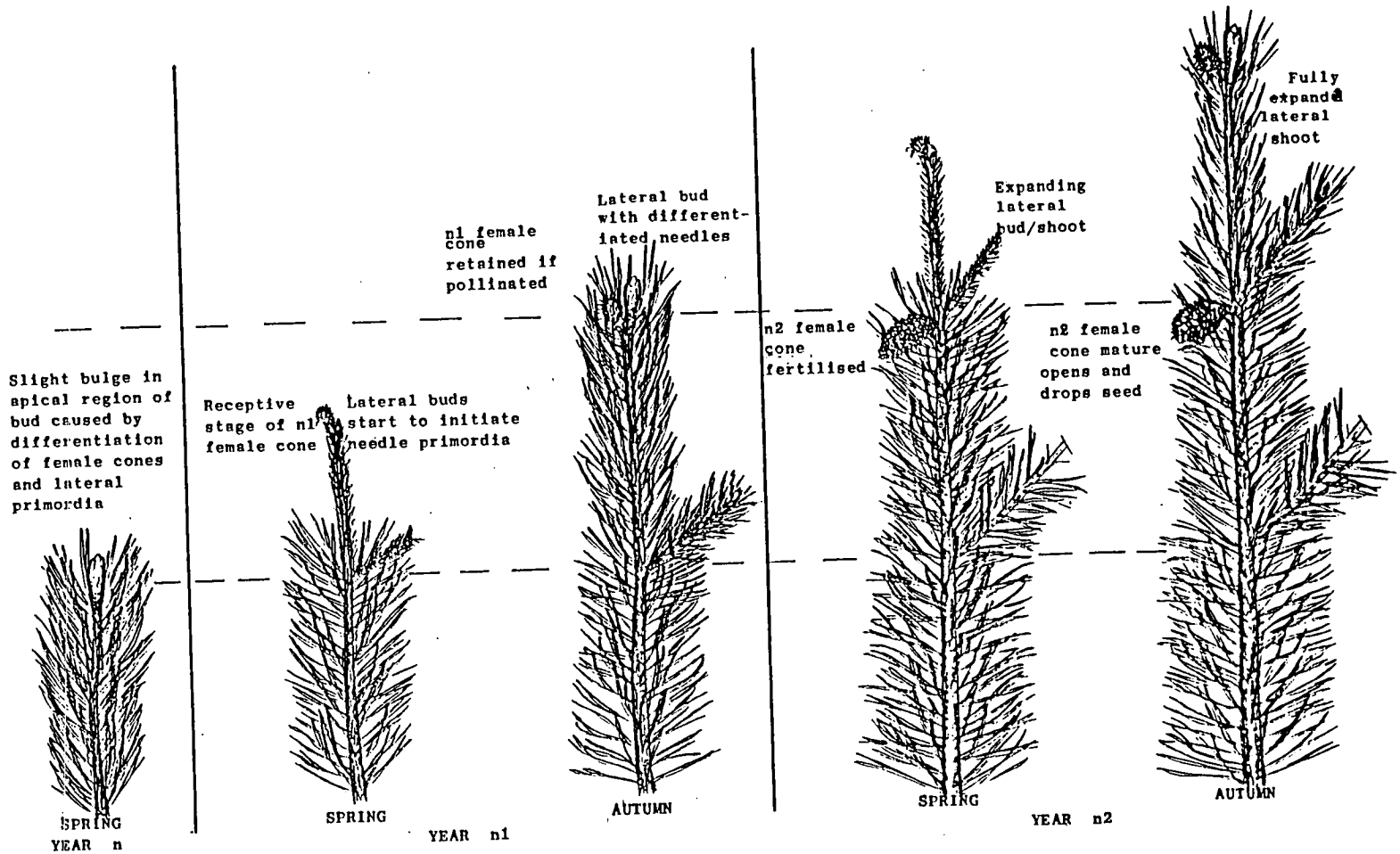


Figure 1.3 Life cycle of vegetative shoots and female cones of P. contorta.

Nevada and the Rocky Mountains. It is one of the most widely harvested forest trees in the west of the U.S.A. because of it's wide distribution and it's value as a source of timber. Its uses include interior panelling, exterior trim, posts, poles and extensive use for pulp and paper. Pinus contorta is considered a 'pioneer' species and is characterized by low shade tolerance, rapid growth in young trees, slow growth in older trees and the ability to grow on almost any forest soil (Owens & Molder 1984). In Britain, this species is a commercial tree which grows well on the poorest upland soils (Zehetmayr 1954, James 1982). In recent years the proportion of Pinus contorta planted has declined in favour of Picea sitchensis largely because of the greater potential production of spruce given adequate nutrition and the greater demand for spruce timber. In addition Panolis flammea (pine beauty moth) has become a major pest requiring large-scale control operations since 1977 (Watt 1988). Pure stands of Pinus contorta are mostly planted on sites which are too poor to sustain Picea sitchensis without heavy fertiliser inputs (Lines 1976).

Three varieties were identified by Critchfield (1957): Pinus contorta var contorta found in coastal regions; var. latifolia found inland on the Rocky mountains; and var. murryana found in the Sierra Nevada. Later studies have suggested there is that a fourth variety var. bolanderi, from the Mendocino white plains in California (see Newman & Jancey 1983, Owens & Molder 1984). In addition, to these varieties each may have female cones which are serotinous (require extreme heat, usually a fire, before they open) or nonserotinous (open in normal dry conditions). There is apparently no difference in the vegetative or reproductive growth between Pinus contorta var. latifolia trees with the two cone types (Muir & Lotan 1985).

1.7 LIFE HISTORY

The growth pattern of the genus Pinus has been divided into ten distinct types (Lanner 1976). One of these the 'contorta' pattern is exemplified by Pinus contorta. This growth habit is based on entirely fixed growth from preformed winter buds, i.e. primordia are

differentiated within a bud which elongates the following year. In some instances there may be several cycles of needles and branch whorls enclosed within the bud (i.e. polycyclic). The factors that cause a bud to become monocyclic or polycyclic are not know. However the vigorous branches in the upper regions of the crown are more likely to form polycyclic buds than the less vigorous branches in the lower crown (Owens & Molder 1984).

In the Pinaceae female cones are normally produced a few years before any male cones (Wareing 1958). Although both sexes are normally found on the same tree, there are distinct patterns of distribution within crowns (Varnell 1977, Powell 1977, Marquard & Hanover 1984, Dick et al. 1985). Female cones occur on longer more vigorous branches in the upper region of the tree crown and are situated on the distal portion of branches. While male cones are more commonly found on the weaker branches, lower in the tree crown.

The processes influencing the differentiation of primordia and elongation of needles and shoots of some north temperate conifers, notably Picea sitchensis and Pinus contorta have been reviewed by Cannell et al. (1976). They concluded that conifer apices are normally mitotically active throughout the year, but that elongation occurs over an eight week period during the summer. They suggested, speaking teleologically, that shoot elongation need not occur throughout the available growing season, because it occurs merely to display the needles to the best advantage at the right time, whereas apical growth needs to occur throughout the year in order to generate as many new needles as possible. The annual growth cycle of Pinus contorta var. contorta and var. latifolia growing in British Columbia was studied by Owens and Molder (1975, 1984). They showed that bud development begins with the initiation of cataphylls in March and that axillary primordia are initiated about two weeks after the subtending cataphylls. Generally Pinus contorta var. contorta differentiates primordia and becomes dormant 2-4 weeks later than Pinus contorta var. latifolia.

Male and female cones of Pinus contorta usually develop in separate buds. However, female cones in amongst male cone clusters have been observed by the author, usually on very prolific trees.

1.7.1 MALE CONES

In British Columbia potential male cones and needle primordia are initiated in Pinus contorta from March through July and differentiation proceeds acropetally along the bud from August to November (Owens and Molder 1975), while in Scotland, differentiation of male cones begin in early July and continues until late-September (Couper 1987). Male cone buds (Figure 1.2a) continue to grow slowly until the following spring when they elongate. As the pollen matures the cones turn from green to yellow or salmon pink and then to brown as they dry out. They shed their very small, light-weight pollen in late May-June depending on the weather (Figure 1.2b). Each male cone contains about half a million pollen grains (Owens & Molder 1984). The male cone-bearing shoot continues to elongate until around midsummer. By this time the basal section of stem, where the male cones were situated is conspicuous because of the lack of needles (Figure 1.2c).

1.7.2 FEMALE CONES

In pines potential female cones and vegetative lateral shoots are usually differentiated at the distal end of developing terminal buds, but a few exceptions have been reported (see Rudolph et al. 1986). In monocyclic buds of Pinus contorta growing in British Columbia, initiation occurs in August with female cones differentiating in October-November (year n) while lateral branch buds do not differentiate until the following spring (Owens & Molder 1975). In central Scotland however, differentiation of the cones occurs much earlier with most female cones differentiated by August although a few develop in September (Couper 1987). Unfortunately, the distinction between monocyclic and polycyclic buds is not clear in her work.

In the spring of the year following differentiation (year n1), female cones elongate and become red or purple, and are receptive to pollen. Large pollination drops are exuded out of the micropyle of the ovules. When pollen comes in contact with the pollination drop it is withdrawn into the micropyle (Owens & Molder 1984). Pollination drops are slightly more viscous than water and contain low concentrations of several sugars. If the cone is pollinated it is usually retained on the branch, otherwise it is aborted (Brown 1971). An increase in auxins following pollination is implicated in the retention of the cone (Brown & Sauve 1975). The pollen tube starts to grow towards the ovules, but progress is arrested by the onset of winter. The pollen tube starts to grow again the following spring (year n2) and fertilisation occurs in early June (Owens & Molder 1984). The cone rapidly increases in size and weight during the spring until about July. During July and August the embryo and seed develop rapidly. The cones are usually mature by September-November (Figure 1.3). Depending on provenance, the cones may shed their seed in dry weather (nonserotinous) or they may require extreme heat (45-50 °C) to destroy the resin which firmly seals the scales of the serotinous cones. Seed may remain viable for several years within sealed serotinous cones.

1.8 OVERVIEW OF RESEARCH PROGRAMME

Following a review of the literature it was decided to measure the the input and output of carbon in both reproductive branches and potted plants and to compare the results with equivalent vegetative branches or plants. This was achieved by the following series of studies:

(i) Male and female cones may alter the morphology of the shoots which bear them and, consequently, their photosynthetic area. The influence of male and female cones on the number of needles and lateral shoots on the branches which bear them was determined in Chapter 2.

(ii) As reproductive structures are a major sink for photo-assimilates they may induce a higher assimilation rate on the shoots which bear them. The influence of cones on the carbon flux of needles immediately adjacent to them was measured, in natural conditions (Chapter 3) and under controlled conditions (Chapter 4).

(iii) Reproductive structures of many species have a reduced respiration rate when illuminated. The respiration rates of male and female cones were measured to determine whether they also contribute significantly to their carbon economy (Chapter 5).

(iv) A small potted plant system was devised to allow a complete model to be constructed for plants with and without cones (Chapter 6). Unfortunately this failed, so the influence of cones on the whole tree was determined by utilizing a simulation model with the parameters set to appropriate values determined in the earlier chapters (Chapter 7).

CHAPTER 2

INFLUENCE OF MALE AND FEMALE CONES ON VEGETATIVE GROWTH

2.1 AIM

Male and female cones may alter the morphology of the shoots which bear them because male cones are formed at the same position as needles within a bud and female cones are formed at the same position as lateral shoot primordia. The aim of this study was to evaluate the morphological differences between vegetative and cone-bearing branches and to estimate the effect of these factors on the growth of trees.

2.2 INTRODUCTION

It is known that female cones are formed at about the same time as lateral shoots within the buds of lodgepole pine, while male cones develop at the same time as needles (Owens and Molder 1975). It has therefore been commonly assumed that female cones replace lateral shoots (Ebell 1971, Cannell 1976, 1979) while male cones replace needles. Thus, cones of both sexes could reduce the photosynthetic area of cone-bearing branches. No data to test this hypothesis could be found in the literature.

In this study the number of needles and lateral shoots produced on vegetative and reproductive 'branch units' were compared. For this purpose, branch units consisting of two years growth (n1 and n2) were taken as the standard unit of comparison. This was done because: (i) the needles on the previous years shoot, the so called parent shoot (Cannell 1976), are known to be the major source of assimilates for the growth in the current year (Clausen & Kozlowski 1967, Gordon & Larson, 1968, Loach & Little 1973, Chung & Barnes 1980, Ericsson 1980), and (ii) the number of needles on the parent shoot is directly related to the number of lateral branches produced the following year (Cannell 1974, 1976).

As male and female cones were collected as two separate studies they will be considered independently in this chapter, and the influence of each will be compared in the final section. Male cones were collected just prior to shedding their pollen in the spring while female cones were collected in the autumn before shedding their seed.

2.3 INFLUENCE OF MALE CONES

2.3.1 MATERIALS

Branch units were collected on 14th to 19th May 1987 from 16 trees growing at the Bush Estate, Penicuik, Scotland (55° 55′ N, 184 m altitude). Four of these trees were 20-year-old and grown from seed, while 12 were 10-year-old rooted cuttings (clones 12 & 13). All branch units were harvested at the same phenological stage, which was just before the male cones shed their pollen. The branch units were removed at the base of the previous year's growth, i.e. they consisted of a parent shoot which elongated in 1986, and which was just beginning to elongate in 1987. The shoots elongating in 1987 will be termed the the current year's growth and consisted of a terminal shoot which was either vegetative or had male cones and lateral shoots which were always vegetative (Figure 2.1) . Branch units with male cones on the terminal shoot will be termed reproductive branch units and those without cones will be termed vegetative branch units.

Two branch units from the same whorl were collected together and considered as a 'pair' when they had parent shoots of similar length, which indicates similar potential for growth (Figure 2.1). Vegetative and reproductive branch units with similar parent length only occurred in the mid-crown region of the tree in four of the genotypes sampled. Therefore, additional reproductive branch units were collected over the whole of the male coning region of these trees and from two others trees, giving a representative sample of male cone-bearing branches in six trees. (Table 2.1).

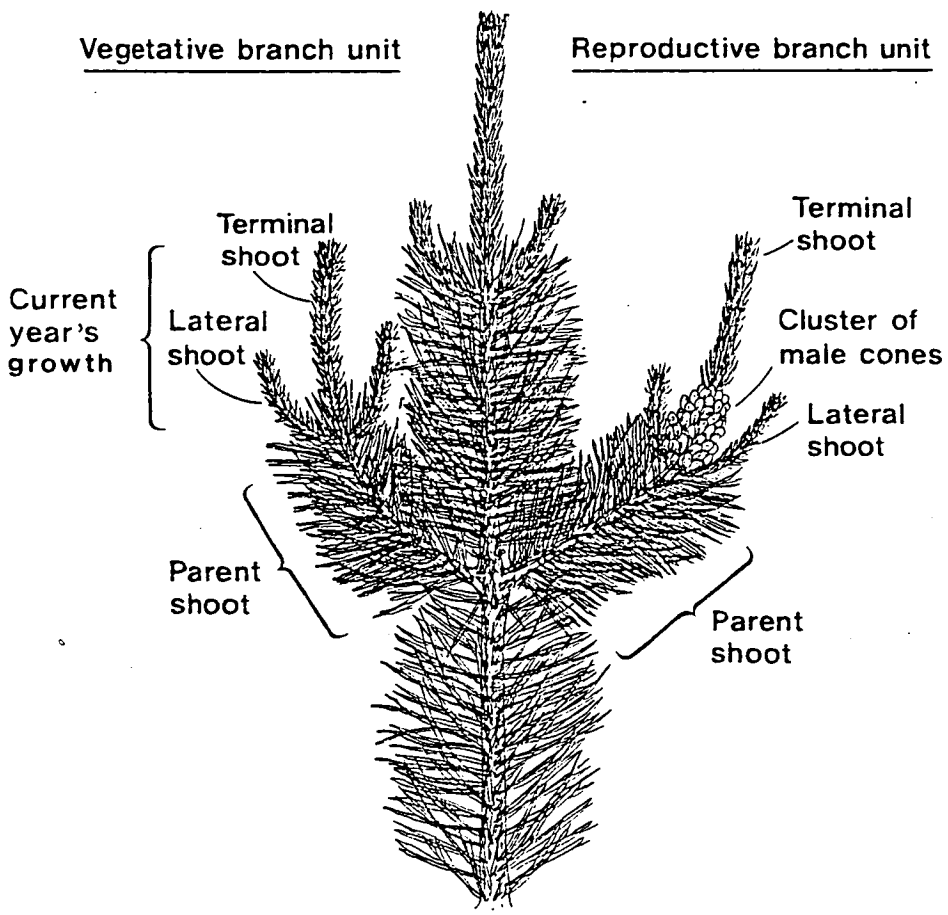


Figure 2.1 Paired vegetative and reproductive branch units arising from the same whorl on P. contorta.

Table 2.1 Number of paired and unpaired, vegetative and reproductive branch units sampled from trees of P. contorta.

Tree	No. Trees	Branch Units: Paired Veg	Paired Male	Unpaired Male	Total Reproductive
2	1	4	4	15	19
12	6	12	12	19	31
13	6	14	14	11	25
8988	1	12	12	14	26
3	1	-	-	31	31
8968	1	-	-	14	14
TOTAL		42	42	104	146

Thus a total of 42 paired vegetative and reproductive branch units were collected from four genotypes (2, 12, 13, 8988), with a further 59 unpaired reproductive branch units. In addition 45 reproductive branch units from two other trees (3, 8968) that did not produce paired vegetative and reproductive branch units were collected.

2.3.2 METHODS

Each morning branches were cut, taken to the laboratory and stored in a cold room at 4 °C for processing that day. The number of lateral shoots on the current year's growth was recorded and the following variables were measured on:

(i) terminal shoot of the current year's growth;
(a) the number of sterile cataphylls, needle pairs and male cones,
(b) dry weight of the terminal shoot, lateral shoots and male cones,
(c) the fresh weight of the male cones,

(ii) parent shoot;
(a) the number of sterile cataphylls and needle pairs,
(b) length and dry weight of stem and
(c) area and dry weight of needle pairs (Figure 2.2).

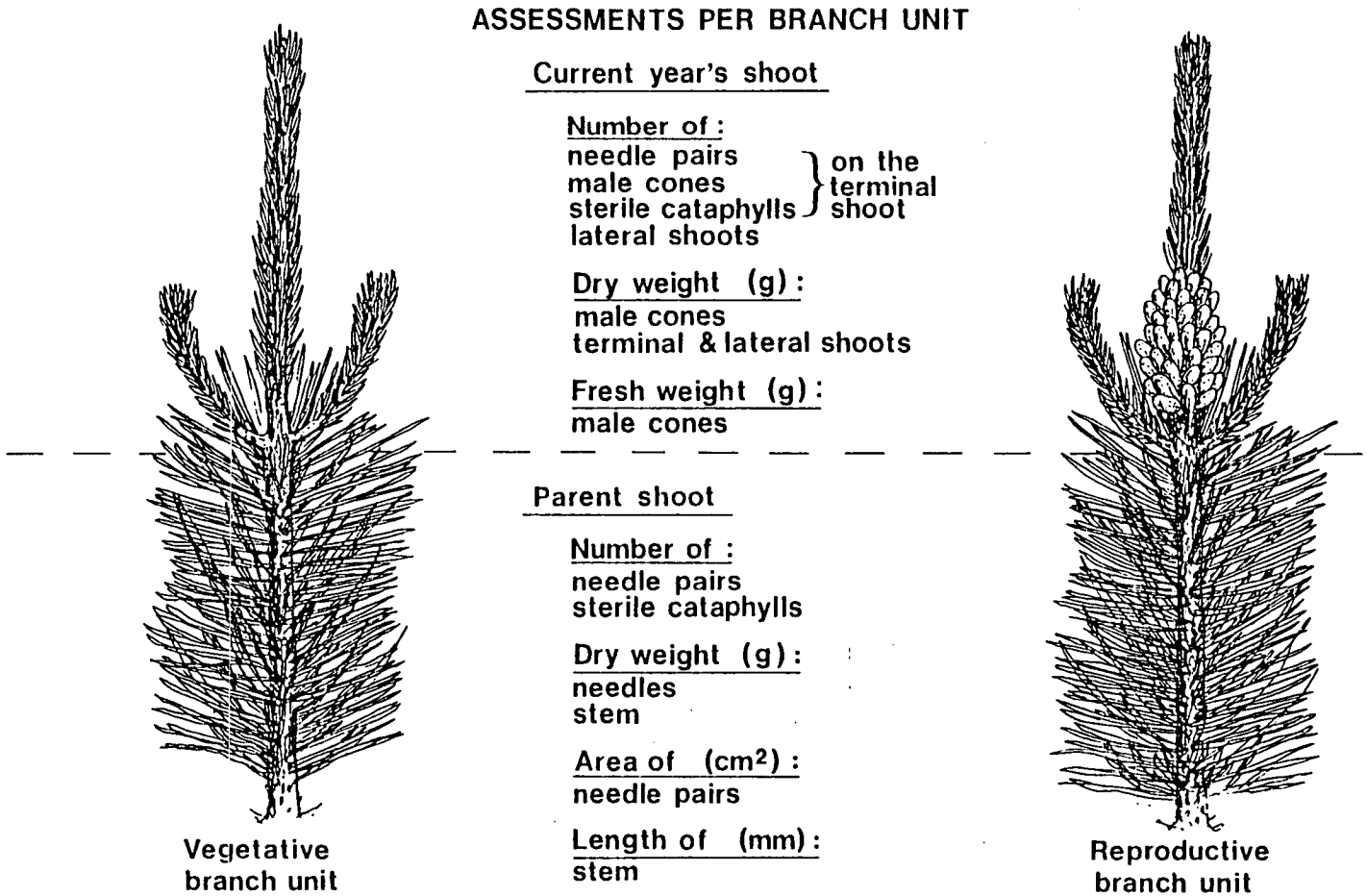


Figure 2.2 Characteristics assessed on vegetative and reproductive branch units of P. contorta.

The weight of the male cones was determined by placing them in pre-dried envelopes (80°C) and subtracting the weight of the envelope. Analysis of variance with a log+1 transformation, was used to determine the difference between vegetative and reproductive branch units for all the variables measured or calculated. The untransformed means are presented for clarity. A stepwise regression (Genstat 5) with a log+1 transformation was used to determine the most important variables contributing to the total dry weight of the current year's growth and to the reduction in the number of needles on the terminal shoot of male cone-bearing branches. A Poisson error distribution was assumed for counts (e.g. number of needles) and a normal error distribution was assumed for continuous variables (e.g. weight of current year's growth).

2.3.3 RESULTS

2.3.3.1 COMPARISON OF PAIRED VEGETATIVE AND REPRODUCTIVE BRANCH UNITS

In this section only the 42 paired vegetative and reproductive branch units are considered. Although there was considerable genotypic variation in all the variables assessed (Table 2.2) it is valid to generalise within this section for all four genotypes because the branch units were paired within each genotype.

Morphological differences

By comparison with the terminal shoot of vegetative branch units terminal shoots with male cones had significantly fewer needle pairs, sterile cataphylls, and total number of differentiated primordia (i.e. needle pairs + sterile cataphylls + male cones). Reproductive branch units also had significantly fewer lateral shoots on the current year's growth compared with vegetative branch units (Table 2.3). There was no significant difference in any of the variables assessed on the parent shoot.

Table 2.2 Mean and one standard error of the mean (Stderr) for variables measured on (i) the current year's growth and (ii) the parent shoot of paired vegetation (veg) and male-cone bearing (male) branch units of P. contorta.

(i) CURRENT YEAR'S GROWTH

		On the terminal shoot									
		Number of Needles		Number of male cones		Number of sterile cataphylls		Total number of Primordia		Number of lateral shoots	
Clone	Branch type	Mean	Stderr	Mean	Stderr	Mean	Stderr	Mean	Stderr	Mean	Stderr
2	Male	121.50	16.64	33.50	8.63	7.50	0.87	162.50	11.51	1.50	0.50
	Veg	164.00	12.73	0.00	0.00	9.00	1.29	173.00	12.56	1.50	0.29
12	Male	103.92	11.01	18.67	4.78	7.42	0.45	130.00	11.42	0.67	0.26
	Veg	127.83	13.51	0.00	0.00	8.92	0.42	136.75	13.78	0.67	0.19
13	Male	57.00	5.17	20.57	1.96	6.14	0.53	83.71	6.35	0.21	0.11
	Veg	97.57	7.85	0.00	0.00	7.57	0.60	105.14	7.55	0.57	0.17
8988	Male	59.92	5.03	27.42	3.36	3.50	0.44	90.83	4.03	0.25	0.13
	Veg	98.25	4.59	0.00	0.00	4.75	0.58	103.00	4.73	0.83	0.17
All	Male	77.38	5.56	23.21	2.03	5.88	0.36	106.48	5.79	0.48	0.11
	Veg	112.74	5.85	0.00	0.00	7.29	0.40	120.02	5.97	0.76	0.10

		Dry weight of terminal shoot (g)		Dry weight of lateral shoots (g)		Dry weight of male cones		Total dry weight of the current year growth		Fresh weight of male cones (g)	
Clone	Branch type	Mean	Stderr	Mean	Stderr	Mean	Stderr	Mean	Stderr	Mean	Stderr
2	Male	0.94	0.14	0.25	0.11	0.69	0.18	1.88	0.35	2.86	0.87
	Veg	1.18	0.13	0.30	0.11	0.00	0.00	1.48	0.25	0.00	0.00
12	Male	0.65	0.11	0.09	0.05	0.48	0.13	1.22	0.20	1.57	0.43
	Veg	0.74	0.14	0.13	0.06	0.00	0.00	0.87	0.20	0.00	0.00
13	Male	0.20	0.03	0.00	0.00	0.51	0.05	0.72	0.07	1.93	0.19
	Veg	0.31	0.04	0.04	0.01	0.00	0.00	0.35	0.05	0.00	0.00
8988	Male	0.40	0.03	0.03	0.02	0.80	0.08	1.22	0.08	3.56	0.37
	Veg	0.52	0.04	0.08	0.03	0.00	0.00	0.61	0.06	0.00	0.00
All	Male	0.46	0.05	0.06	0.02	0.60	0.05	1.12	0.09	2.38	0.22
	Veg	0.58	0.06	0.10	0.02	0.00	0.00	0.68	0.08	0.00	0.00

(Continued)

Table 2.2 (Cont'd)

(ii) PARENT SHOOT

		Stem length (mm)		Stem dry weight (g)		Number of Needle pairs		Needle area (cm^2)		Needle dry weight (g)		Number of sterile cataphylls		Total number of primordia	
Clone	Branch type	Mean	Stderr	Mean	Stderr	Mean	Stderr	Mean	Stderr	Mean	Stderr	Mean	Stderr	Mean	Stderr
2	Male	173.50	10.72	1.72	0.29	112.25	12.33	139.84	21.24	5.96	0.94	11.50	1.44	123.75	0.87
	Veg	169.75	20.10	1.76	0.39	95.50	20.04	113.92	23.65	4.92	0.98	11.75	1.70	107.25	0.00
12	Male	120.08	13.59	0.87	0.17	95.83	12.01	98.35	15.22	3.77	0.61	8.25	0.91	104.08	0.43
	Veg	116.92	13.65	0.74	0.15	83.58	11.58	91.33	14.49	3.55	0.59	10.00	0.94	93.58	0.00
13	Male	49.57	5.17	0.30	0.05	84.14	9.25	47.21	6.85	1.69	0.24	7.93	0.49	92.07	0.19
	Veg	55.21	7.06	0.38	0.06	85.21	10.39	55.63	8.26	2.07	0.31	8.36	0.69	93.57	0.00
8988	Male	78.58	4.23	0.47	0.04	70.08	5.17	72.18	7.13	3.14	0.31	7.00	0.84	77.08	0.37
	Veg	87.58	6.26	0.63	0.07	81.58	5.69	81.87	6.33	3.67	0.29	6.83	0.69	88.42	0.00
A11	Male	89.81	7.47	0.65	0.09	86.14	5.22	77.78	7.03	3.11	0.29	8.10	0.44	94.24	0.22
	Veg	93.00	7.41	0.69	0.08	84.69	5.23	78.88	6.22	3.22	0.26	8.71	0.48	93.40	0.00

Table 2.3 Mean values of 10 morphological variables assessed on paired vegetative and reproductive branch units of P. contorta (SE = standard error of the mean).

Variable	Branch Units	
	Vegetative (SE)	Reproductive (SE)
(1) CURRENT YEARS SHOOT		
number of :		
terminal needle pairs	112.7 (5.85)	77.4 (5.56)
terminal sterile cataphylls	7.3 (0.4)	5.9 (0.36)
male cones	0	23.2 (2.03)
total differentiated primordia	120.0 (5.97)	106.5 (5.79)
lateral shoots	0.8 (0.1)	0.5 (0.11)
(2) PARENT SHOOT		
number of :		
needle pairs	84.7 (5.23)	86.1 (5.22)
sterile cataphylls	8.7 (0.48)	8.1 (0.44)
total differentiated primordia	93.4 (5.25)	94.2 (5.27)
stem length (mm)	92.0 (7.4)	89.8 (7.5)
total needle area (cm2)	78.9 (7.03)	77.8 (6.22)

The reduction in the total number of differentiated primordia on the terminal shoot of reproductive branch units was proportional to the total number of differentiated primordia found on the terminal shoot of vegetative branch units, as shown in Figure 2.3 for which

$$N_{pm} = 7.2 + 0.827\ N_{pv} \qquad (2.1)$$

where

N_{pm} = total number of differentiated primordia on male cone-bearing shoots

N_{pv} = total number of differentiated primordia on vegetative shoots

	COEFFICIENT	t-RATIO	
CONSTANT	7.24	0.72	ns
SLOPE	0.827	10.36	p <0.001

r^2 0.72

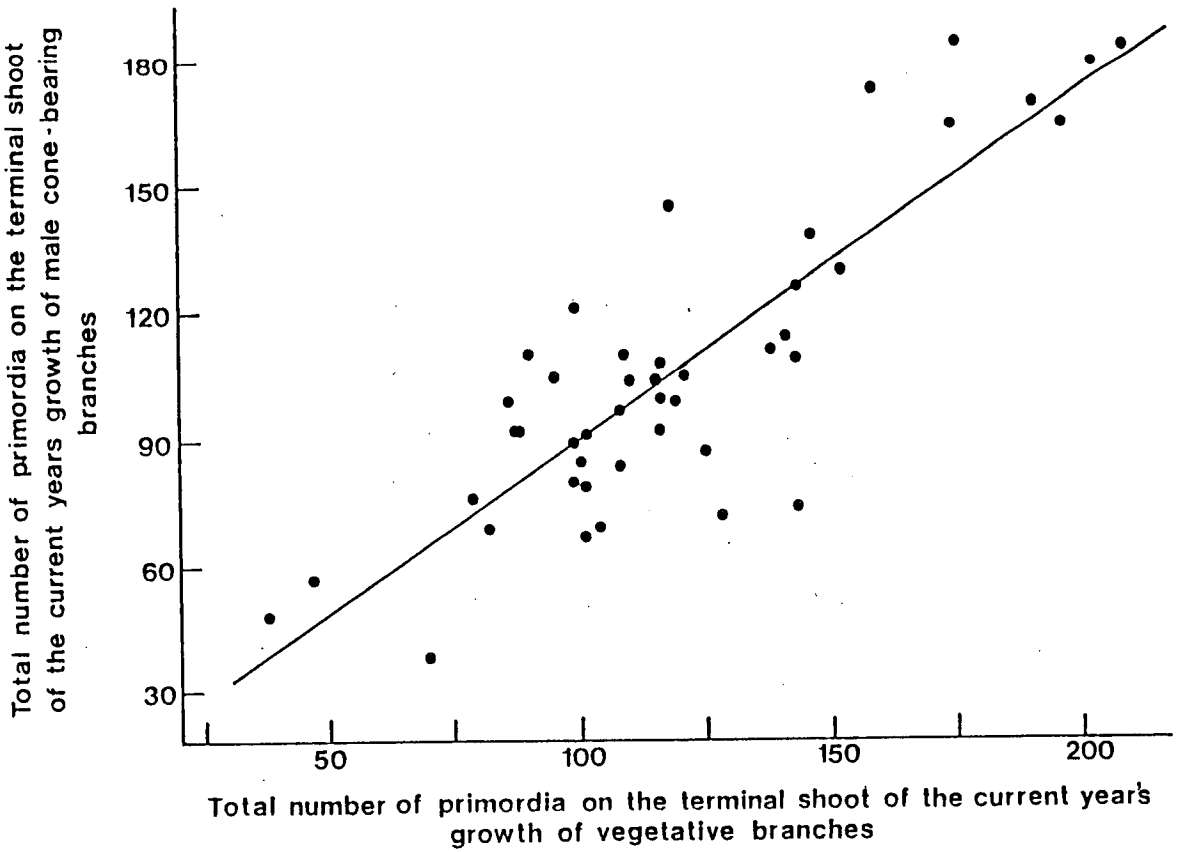


Figure 2.3 The relationship between the number of primordia on the terminal shoot of the current year's growth of paired vegetative and male cone-bearing branches of P. contorta.

The number of needles on the terminal shoot of the current year's growth of vegetative branch units was also linearly related to the number of needles on the current year's shoots of reproductive branch units as shown in Figure 2.4 for which

$$N_{nm} = -8.2 + 0.759 \; N_{nv} \qquad (2.2)$$

N_{nm} = Number of needles on male cone-bearing shoots
N_{nv} = Number of needles on vegetative shoots

	COEFFICIENT	t-RATIO	
CONSTANT	-8.17	-0.76	ns
SLOPE	0.758	8.46	p <0.001

r^2 0.62

A stepwise regression was used to determine which variables could be related specifically to the reduction in the number of needle pairs on the terminal shoot. For logistical reasons the original pairs of branch units were treated as experimental blocks and fitted first in the model. The following factors were then included in the stepwise regression model:

(i) type of branch unit;

(ii) current year's growth,
(a) the number of male cones,
(b) number of lateral shoots,

(iii) parent shoot;
(a) number of needles,
(b) needle dry weight,
(c) needle surface area,
(d) length and weight of the parent stem.

The model accounted for 86.4% of the variance (Table 2.4).

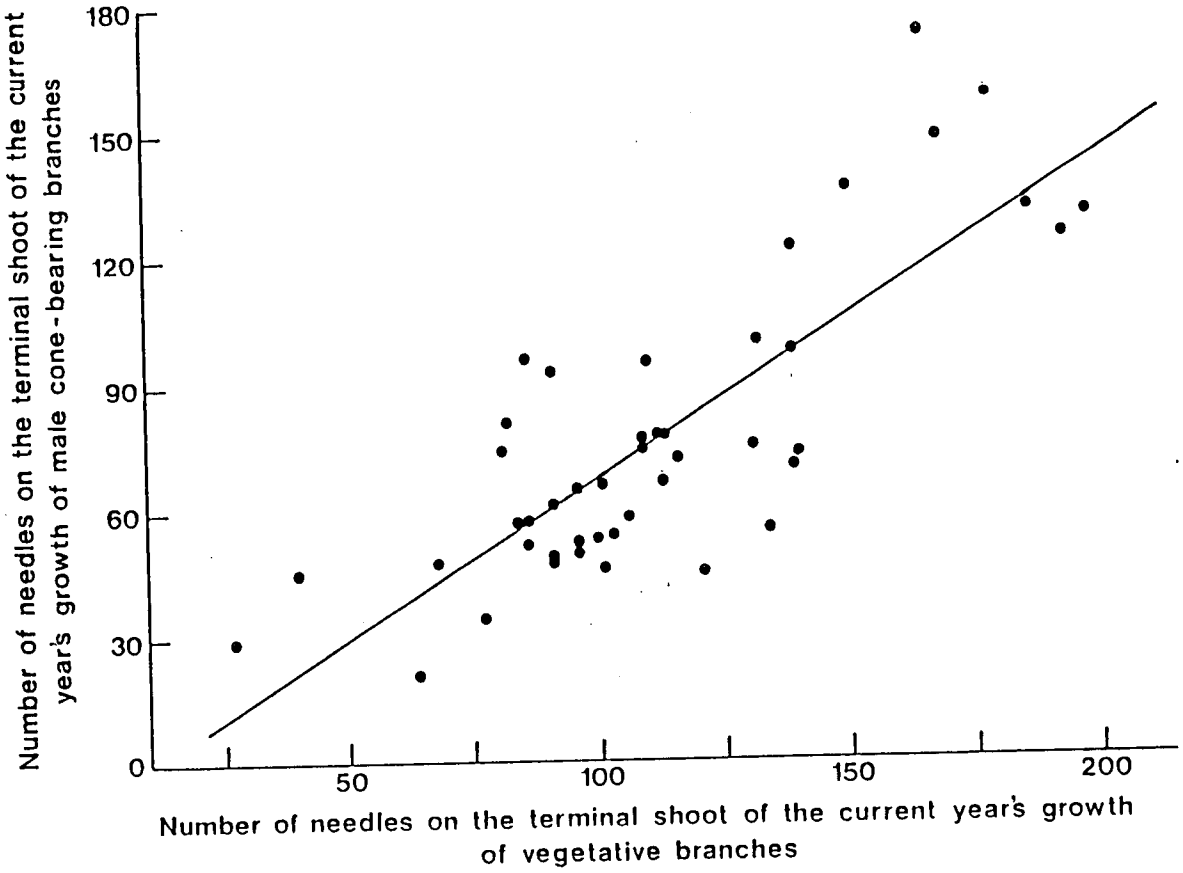


Figure 2.4 The relationship between the number of needles on the paired vegetative and male cone-bearing branches on the terminal shoot of the current year's growth of P. contorta.

Table 2.4 Analysis of variance with a log+1 transformation for the stepwise regression to determine the variables related to the reduction in the number of needles on the terminal shoot of the current year's growth of vegetative and reproductive branch units (OF= order the terms were fitted to the model, DF = degree of freedom, SS = sum of squares and P = probability value, sign of the regression coefficient in brackets).

Terms	OF	DF	SS	Mean SS	Variance ratio	P value	
shoot pair	1	41	12.43789	0.30336	11.44	<0.01	
shoot type	5	1	0.03966	0.03966	1.50	ns	
(i) CURRENT YEARS SHOOT							
Number of:							
male cones	4	1	2.84945	2.84945	107.42	<0.001	(-)
lateral shoots	9	1	0.98949	0.98949	0.47	ns	
(ii) PARENT SHOOT							
weight per needle	2	1	1.28578	1.28578	48.47	<0.001	(+)
number of needles	3	1	0.16096	0.16096	6.07	<0.05	(+)
area per needle	6	1	0.00006	0.00006	0.00	ns	
stem weight	7	1	0.00326	0.00326	0.12	ns	
stem length	8	1	0.00276	0.00276	0.10	ns	
residual		34	0.00381	0.00012			

The number of male cones formed had the largest influence on the number of needles occurring on the terminal shoot of the current year's growth. The only other significantly associated variables were the number and weight of needles on the parent shoot.

Dry weight allocation

Reproductive branch units, on average, had almost twice as much dry weight as vegetative branch units in the current year i.e. terminal + lateral shoots (Figure 2.5). On reproductive branch units 95% of the total dry weight was allocated to the terminal shoot and 5% to the lateral shoots. Male cones contributed 54% of the dry weight of the terminal shoot. By comparison, on vegetative branch units 85% of the total dry weight was allocated to the terminal shoot and 15%

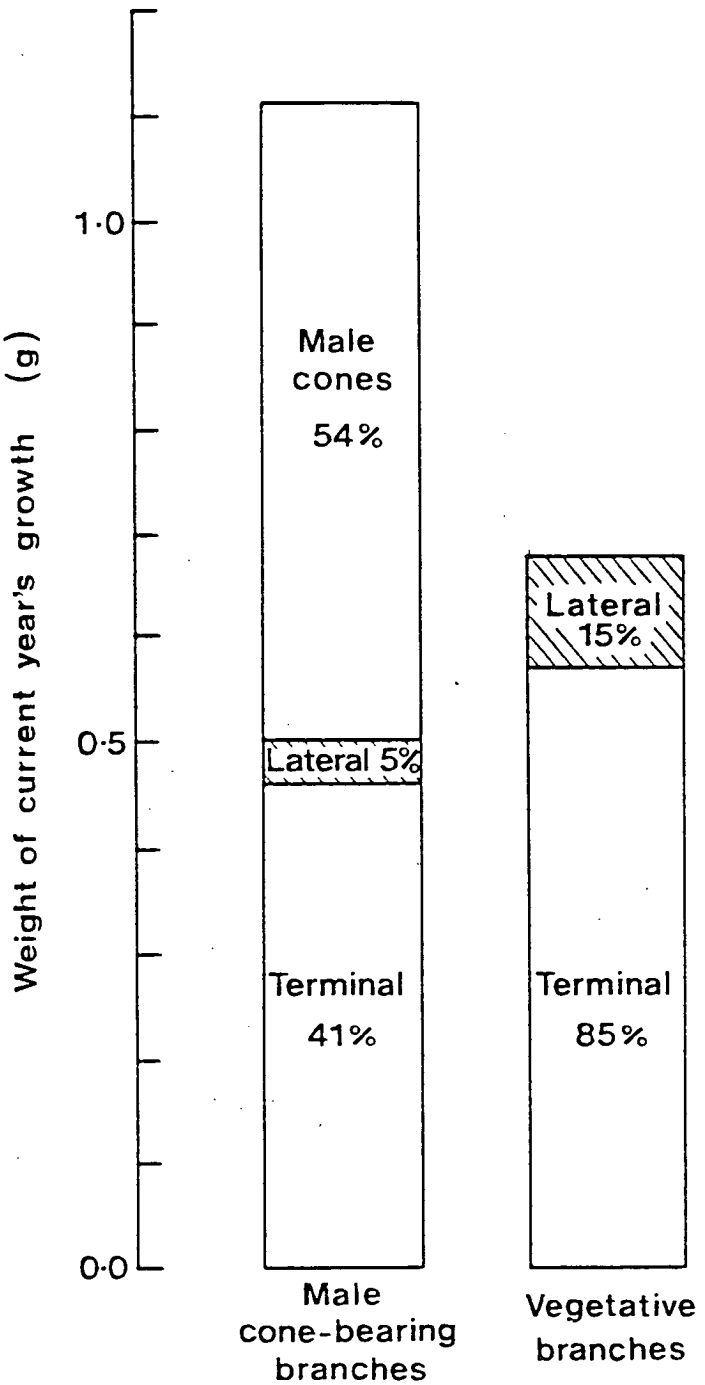


Figure 2.5 Total weight in May of the current year's growth of 42 vegetative and male cone-bearing branches of P. contorta.

to the lateral shoots. Male cones therefore significantly alter the allocation coefficients of shoots which bore them. The allocation coefficients varied slightly between trees (Figure 2.6a). Genotypes with the least total dry weight had the largest proportion of male cones, and the smallest proportion allocated to lateral shoots (Figure 2.6b).

On the parent shoot the dry weight per needle was the only significantly different weight measured between the two types of branch units (Table 2.5).

Table 2.5 Mean weights of the needles, shoots and cones of paired vegetative and reproductive branch units of P. contorta. (SE = standard error of the mean).

	Branch Unit	
Variable	Vegetative (SE)	Reproductive (SE)
(1) CURRENT YEAR'S SHOOT		
dry weight (g) :		
terminal shoot	0.575 (0.06)	0.456 (0.05)
lateral shoots	0.102 (0.02)	0.059 (0.02)
male cones	0	0.603 (0.05)
total dry weight	0.677 (0.08)	1.118 (0.09)
fresh weight of male cones (g)	0	2.384 (0.22)
(2) PARENT SHOOT		
dry weight (g) :		
stem	0.687 (0.08)	0.647 (0.09)
total needles	3.222 (0.26)	3.106 (0.29)
weight per needle (g needle-1)	0.037 (0.00)	0.035 (0.00)
weight per unit stem length (g mm-1)	0.007 (0.00)	0.006 (0.00)

The reduction in individual needle weight on the parent shoots of reproductive branch units however, does not account for all the difference in total dry weight between the reproductive and vegetative branch units (0.441 g). The mean decrease in needle weight on the parent shoot per paired branch was 0.1871 g (Equation 2.3).

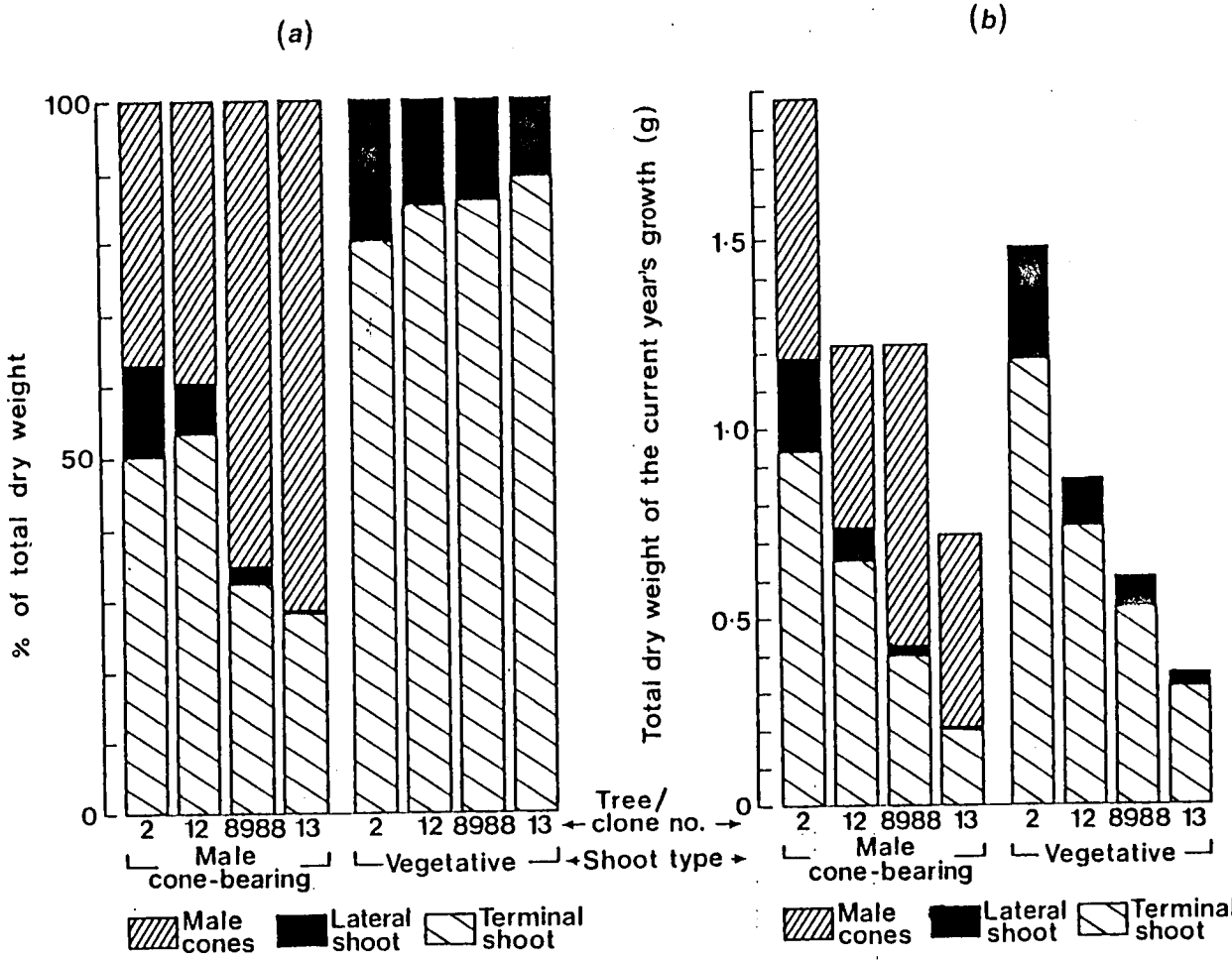


Figure 2.6 Variation in a) the percentage and b) the total dry weight of the current year's growth of paired vegetative and male cone-bearing branches of P. contorta.

$$(W_{nm} - W_{nv})\ N_{nm} = -0.187\ g \qquad (2.3)$$

W_{nm} = weight per needle on the parent shoot of male cone-bearing branch units,

W_{nv} = weight per needle on the parent shoot of vegetative branch units

and N_{nm} = number of needles on the parent shoot of male cone-bearing branch units.

Reproductive branch units, therefore, had on average 0.254 g (0.441 - 0.187 g) of additional dry weight compared with vegetative branch units. This is approximately 23% of the current year's dry weight.

A stepwise regression was used to determine which variables were significantly associated with the total dry weight in the current year. The following factors were included in the stepwise regression model:

(i) type of branch unit;

(ii) current year's growth;
(a) number of male cones,
(b) number of terminal needles,
(c) number of lateral shoots,

(ii) parent shoot;
(a) number of needles,
(b) weight and surface area per needle and
(c) stem length and weight.

The model accounted for 89% of variance (Table 2.6) .

The number of male cones and the number of needles on the terminal shoot had a large influence in determining the total dry weight produced in the current year. The number of lateral shoots was not significant. On the parent shoot the weight per needle was positively associated with the total dry weight of the shoots produced in the current year. However the stem length had a weak

negative relationship with the total dry weight produced in the current year. This negative relationship accounted for only a small percentage of the variance and was not apparent when the untransformed data were plotted (Figure 2.7).

Table 2.6 Analysis of variance with a log+1 transformation of a stepwise regression for the relationship of 10 variables with the total dry weight of the current year's growth (OF = order the terms were fitted to the model, DF = degrees freedom SS = sum of squares and P = probability value, sign of the regression coefficient in brackets).

Terms	OF	DF	SS	Mean SS	Variance ratio	P value	
shoot pair	1	41	5.023711	0.122530	13.36	<0.001	
shoot type	4	1	0.100834	0.100834	11.00	<0.01	
(i) CURRENT YEAR'S SHOOT							
Number of :							
male cones	2	1	1.326069	1.326069	144.62	<0.001	(+)
terminal needles	3	1	0.100053	0.100053	10.91	<0.01	(+)
lateral shoots	10	1	0.004600	0.004600	0.50	ns	
(ii) PARENT SHOOT							
weight per needle	5	1	0.064031	0.064031	6.98	<0.05	(+)
stem length	6	1	0.043058	0.043058	4.70	<0.05	(-)
area per needle	7	1	0.011941	0.011941	1.30	ns	
number of needles	8	1	0.000588	0.000588	0.06	ns	
stem weight	9	1	0.008567	0.008567	0.93	ns	
residual		36	0.330101	0.011941			

2.3.3.2 INFLUENCE OF MALE CONES ON WHOLE TREES

As the paired branch units were restricted to the intermediate zone between the male and female cone producing regions of the tree, extra branch units were also collected over the whole tree. However when this larger data set was compared with that of the paired branch units the only significantly different variables were the number of sterile cataphylls (unpaired shoots had 1 less) and the length of the parent shoot (unpaired were 8 mm shorter). This

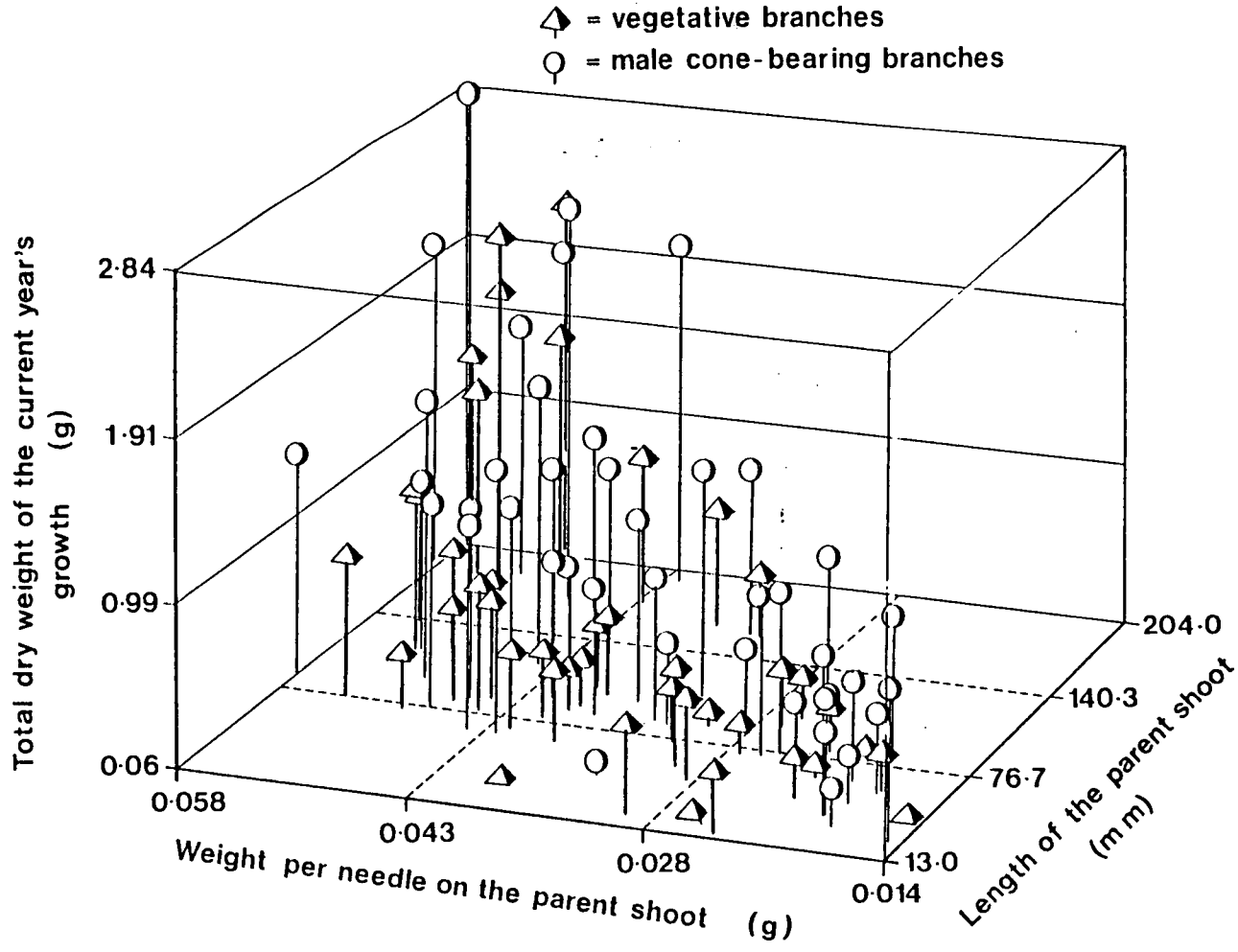


Figure 2.7 Relationship between the total weight of the current year's growth, weight per needle on the parent shoot and length of the parent shoot on P. contorta.

indicates that the restricted area was in fact fairly representative of the whole tree.

Reproductive branch units from two additional trees were also collected to give a wider genetic sample. There was variation in all the variables measured in this study (Table 2.7). However, despite a two-fold difference amongst trees in the total dry weight contained in the current year's growth (Figure 2.8b), all trees allocated dry weight between the structures of the current year's growth in the same order (Figures 2.8a). The dry weight allocated to lateral shoots was the the most variable component of the current year's growth. In proportional terms, tree number two produced nine times more dry weight than trees of clone 13.

All 146 branch units representing the four trees and two clones studied were utilised to predict the effect of male cone formation on the number of needles on whole trees. It is considered valid to do this although the equations utilized were only determined on the paired data sets because the two clones not represented in the paired analyses had branch characteristics similar to the other four genotypes (Table 2.7). Furthermore the equations fitted to the paired data set are considered valid for all the branch units collected as the length of parent shoot was the only significantly different variable between the paired and unpaired data sets. Therefore the function determined in section 2.3.3.1 (equation 2.2) was used to estimate the number of needles that male cone-bearing shoots would have formed if they had not produced male cones as follows:

$$\text{estimated number of needles} = (8.2 + N_{nm})/0.759 \qquad (2.4)$$

where N_{nm} = number of needles on the terminal shoot of male cone-bearing branch units.

This was then rearranged assuming that the difference between the estimated number of needles and the actual number represented the number of needles lost as a result of the formation of male cones.

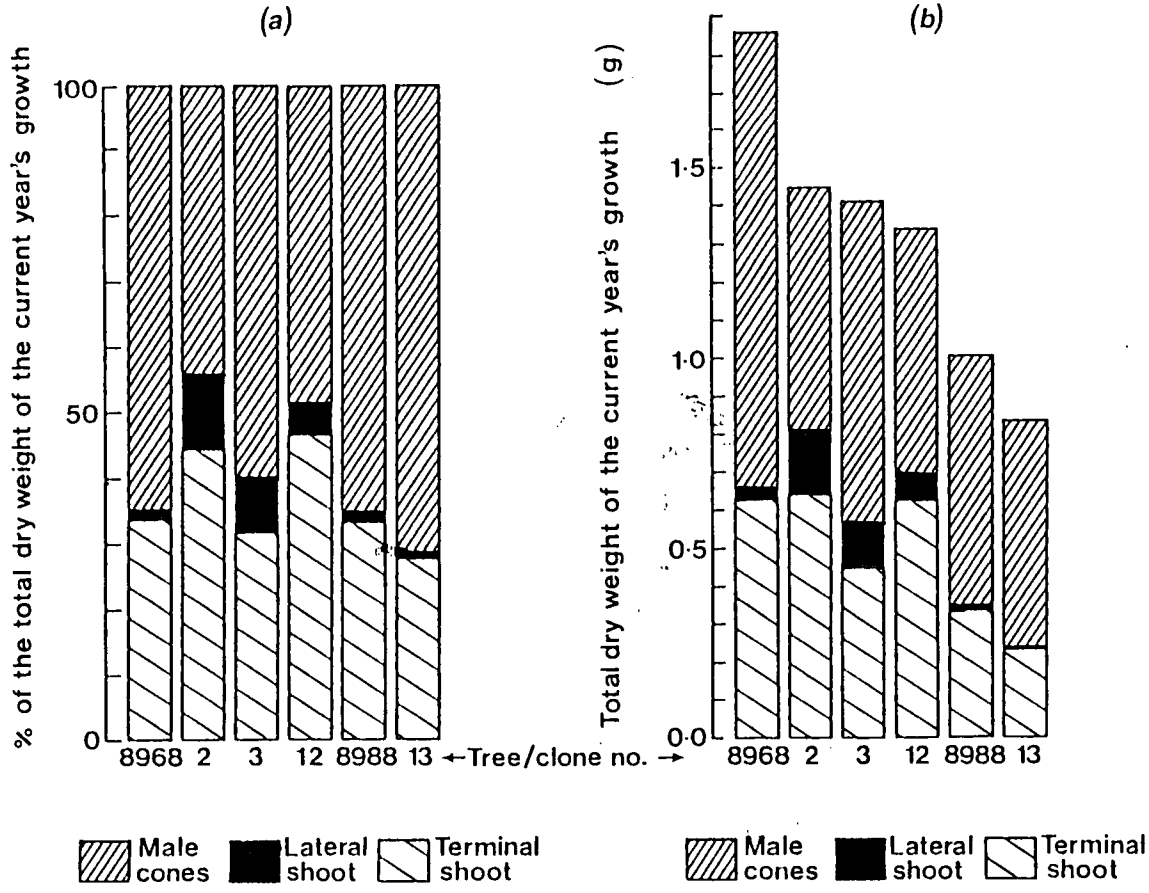


Figure 2.8 Variation in a) the percentage and b) the total weight of the current year's growth of male cone-bearing branches sampled throughtout the whole male cone-bearing region of P. contorta tree crowns.

Table 2.7 Mean and standard error of the mean for variables measured on (i) the current year's growth and (ii) the parent shoot of vegetative and male-cone bearing branches of P. contorta.

(i) CURRENT YEAR'S SHOOTS

		Trees					
		2	3	12	13	8968	8988
Number of needles on terminal shoot	Mean	101.68	56.00	96.19	60.52	65.93	53.23
	Stderr	5.97	4.59	6.89	4.79	4.19	3.71
Number of male cones	Mean	34.53	34.23	23.87	23.44	50.64	24.38
	Stderr	4.12	2.44	2.79	1.87	2.93	2.69
Number of sterile cataphylls on terminal shoot	Mean	7.53	7.71	8.32	5.36	7.21	2.96
	Stderr	0.57	0.46	0.46	0.38	0.73	0.23
Total number of differentated Primordia on terminal shoot	Mean	143.74	97.94	128.39	89.32	123.79	80.58
	Stderr	5.27	5.59	7.57	5.72	6.77	3.43
Number lateral shoots	Mean	1.05	0.84	0.58	0.24	0.14	0.15
	Stderr	0.19	0.24	0.15	0.10	0.10	0.07
Dry weight of terminal shoot (g)	Mean	0.64	0.45	0.62	0.23	0.62	0.33
	Stderr	0.06	0.05	0.07	0.03	0.08	0.02
Dry weight of lateral shoots (g)	Mean	0.17	0.12	0.07	0.01	0.03	0.02
	Stderr	0.04	0.05	0.03	0.00	0.03	0.01
Dry weight of male cones (g)	Mean	0.64	0.84	0.65	0.59	1.20	0.66
	Stderr	0.08	0.07	0.08	0.05	0.13	0.07
Total dry weight of current year's growth	Mean	1.45	1.41	1.34	0.83	1.86	1.01
	Stderr	0.13	0.13	0.13	0.07	0.22	0.07
Fresh weight of males cones (g)	Mean	2.34	2.99	2.06	2.32	5.38	2.93
	Stderr	0.33	0.22	0.25	0.21	0.51	0.30

(Continued)

Table 2.7 (Cont'd)

(ii) PARENT SHOOT

		Trees					
		2	3	12	13	8968	8988
Stem length (mm) needles	Mean	134.05	115.74	107.26	50.52	78.71	67.96
	Stderr	9.19	7.34	8.43	3.74	8.78	3.88
Dry weight of stem (g)	Mean	1.11	0.97	0.81	0.34	0.74	0.37
	Stderr	0.14	0.15	0.12	0.05	0.15	0.03
Number needles pairs	Mean	85.26	73.48	87.26	95.32	67.00	59.04
	Stderr	9.25	6.72	9.08	8.04	10.62	4.19
Needle area (cm^2)	Mean	103.74	87.36	92.18	55.91	92.53	58.31
	Stderr	11.71	8.71	12.05	6.47	14.10	5.10
Needle weight (g)	Mean	4.34	4.18	3.69	2.00	3.50	2.54
	Stderr	0.51	0.48	0.49	0.24	0.58	0.22
Number sterile cataphylls	Mean	7.63	8.84	8.61	7.64	11.07	5.77
	Stderr	0.73	0.51	0.51	0.48	1.02	0.53
Total number differentated Primordia	Mean	92.89	82.32	95.87	02.96	78.07	64.81
	Stderr	9.44	7.06	9.15	8.11	11.15	4.30

Thus:

$$\text{number of needles lost} = ((8.2 + N_{nm})/0.759) - N_{nm} \qquad (2.5)$$
$$= 10.8 + 0.32\ N_{nm}.$$

Consequently it was estimated that as a result of male cone production between 16 and 72 needles had been lost per branch, depending on the genotype (Table 2.8). This equates to between 27% and 50% of the estimated number of needles.

Table 2.8 Estimated number of needles and % of the total needle complement lost per current year's terminal shoot as a result of male cone production in P. contorta.

Tree	Minimum		Maximum		Mean		Standard error
	Number	%	Number	%	Number	%	of the mean
2	33	28%	58	32%	43	30%	1.9
12	25	27%	72	36%	42	31%	2.2
13	16	30%	46	50%	30	35%	1.5
8988	19	30%	43	42%	28	35%	1.2
3	18	29%	51	44%	29	35%	1.5
8968	24	30%	43	36%	32	33%	1.3
ALL TREES	16	27%	72	50%	33	33%	0.9

2.3.4 DISCUSSION

In P. contorta the presence of male cones appears to alter assimilate production and allocation by: (i) reducing the number of needles per branch,(ii) producing smaller and lighter needles on the parent shoot, and (iii) inhibiting the number of vegetative lateral shoots which grow on reproductive branch units.

Males cones were found to reduce the number of needles formed on the branches which bear them by an estimated 33% per branch over the whole male coning region of a tree. This reduction in the number of needles may not have a directly proportional effect on the total assimilate production of the tree because: (i) the male cone-bearing branches on pine are mainly on the lower, relatively less productive regions of the tree crown (Wareing 1958, Hashizume 1979) and (ii) after the male cones have abscised, there may be more light

penetration into the canopy and potentially a higher photosynthetic rate, of the remaining needles. This may, to some extent, compensate for the lack of needles. In this context it may be significant that male cones are usually not found on young trees until they are 10 to 15 year's old (Wareing 1958) by which time it may be supposed that there would be fairly strong attenuation of light within the tree crown. The magnitude of the reduction in needle area found in this study would suggest that the reproductive state of the individual trees should be considered when estimating foliage area from sapwood area as demonstrated by Hungerford (1987).

Male cones however did not simply replace needles on the branches which bore them. A 6 to 18% reduction in the total number of differentiated primordia (i.e. number of needle pairs + number of male cones + sterile cataphylls) was found on the terminal shoot of paired vegetative and reproductive branch units. This reduction may occur because early differentiation of primordia to form male cones reduces both assimilates and plant growth regulators available at the shoot apex so leading to the initiation of fewer primordia. This hypothesis is consistent with the initiation of male cones at the base of the developing bud and the differentiation of primordia in a wave proceeding from the base to the tip of the bud as described Owens and Molder (1975)

The needles that are lost as a result of male cone production are known from defoliation experiments to be the most important for vegetative growth i.e. the current year's needles, (see Ericsson 1980). A major storage area for carbohydrates and nitrogen is also significantly reduced, (Kulman 1965, Krueger 1967). However the reduction in needle number occurs early in the growing season and this, if equivalent to early season defoliation, would not be as detrimental to growth as needles lost later in the season (Ericsson 1980). It is extremely noticeable that once a branch system starts to produce male cones this continues every year. It is likely, therefore, that the defoliation effects associated with male cone production may accumulate and become significant. Unfortunately no equivalent vegetative and reproductive branch systems could be found on which to measure the long term effect of cone production.

Reproductive branch units had significantly fewer lateral shoots compared with vegetative branch units. These lateral shoots were initiated the year before the male cones but differentiate and grew at the same time as the male cones (Owens and Molder 1975). It is probable, therefore, that the number of lateral buds initiated was similar in both vegetative and reproductive branch units and that the reduction in lateral shoots arose from the suppression of the buds by the developing male cones. Unfortunately, the occurrence of these inhibited or latent lateral buds was not noted.

Assimilates stored over winter, or assimilated in the early spring are used in the initial elongation phase of the current year's growth (Rutter 1957, Clausen and Kozlowski 1967, Kimura 1969, Little 1970a, Kozlowski 1971a, Olofinboba and Kozlowski 1973). Therefore a negative relationship between the total dry weight of the current year's growth and the weight of individual needles on the parent shoot might have been expected in the present study. This would indicate the transfer of material from the needles on the parent shoot to the current elongating shoots. Although such a negative relationship was not observed in this study the significant reduction in the dry weight per needle on parent shoots of reproductive branch units, compared with vegetative branch units, supports the above suggestion, thereby indicating that male cones are indeed a larger drain on the parent shoot of reproductive branch units than the vegetative shoots are on vegetative branch units.

At first sight the above observations seems to be inconsistent with the finding that the total dry weight of the current year's growth, on both vegetative and reproductive branch units was positively related to the weight of needles on the parent shoot (i.e. branches with the largest dry weight of current tissue also had the largest weight per needle on the parent shoot). This apparent inconsistency may be explained if it is assumed that the photosynthetic rate of the parent needles was proportional to the strength of the photosynthate sink, i.e. the number of male cones, terminal and lateral needles (see review by Neales and Incoll 1968 and Bidwell 1983). As these structures are differentiated the previous summer

(Owens and Molder 1975) it may be supposed that assimilates would be stored over winter in the parent needles, resulting in a springtime weight per needle proportional to the subsequent number of male cones and needles on the expanding terminal and lateral shoots. The parents shoots of reproductive branch units have been found (see Chapters 3 and 4) to have a significantly higher autumn rate of photosynthesis compared to vegetative branch units further supporting this explanation.

A weak negative relationship was found between the total dry weight of the current year's growth and the length of the parent shoot. This weak relationship was not apparent when the data were plotted but only after the variance attributable to the number of male cones and terminal needles was removed. This may result from competition between the needles on the parent shoot and the developing buds for assimilates and plant growth regulators. The parent shoot elongates at the same time as the newly formed terminal and lateral buds are differentiating primordia for the next season (Owens and Moulder 1975, Couper 1987).

Compared to equivalent vegetative branch units those with male cones had on average 23% more dry weight. Although some of this may have been imported from surrounding branches the male cones refix a percentage of their respiratory CO2 and thus contribute to their own carbon economy (see Chapter 5). This phenomenon has also been observed in female cones of pine and spruce (Dickmann and Kozlowski 1970a, Linder and Troeng 1981, Koppel et al. 1987).

Reproductive branch units allocated approximately 45-65% of the total dry weight of the current growth into male cones, indicating their importance as major photosynthate sinks. On both reproductive and vegetative branch units the weight of the terminal shoot was always larger than that of the lateral shoots. This dominance of the terminal shoot over the lateral shoots results in the very characteristic shape of pine trees. A reduction in stem elongation and needle area on a whole branch as a result of male cone production can not be determined from this study, as the vegetative growth was not complete at the time of sampling. An equivalent set

of paired data would need to be collected at the end of the growing season, but this was not possible in the present study because of a shortage of suitable plant material.

In conclusion, male cones significantly reduced the number of needles on the branches which bore them and in the spring altered the allocation of dry weight within the branch unit. It is questionable whether the compensatory mechanisms discussed in this chapter: (i) increased light penetration to the lower needles, (ii) increased photosynthetic rate of parent needles resulting in increased storage of assimilates over winter and in the early spring, and (iii) refixation of the male cones own respiratory CO_2, could completely compensate for the presence of male cones on a branch. These factors are investigated in the following chapters.

2.3.5 SUMMARY

1. Shoots bearing male cones have up to 60% fewer needle pairs than equivalent vegetative shoots, and a significant reduction in the total number of differentiated primordia (ie. male cones + needle pairs + sterile cataphylls). This indicates that the cones do not simply replace needles.

2. It was estimated that, on whole trees, male cones result in a 27% - 50% reduction in the number of needles per branch.

3. In early spring, branches bearing male cones produced on average 0.441 g more dry weight than vegetative branches. On average 95% of the dry weight was allocated to the terminal shoot (54% of which was male cones) and 5% allocated to the lateral shoots. By comparison vegetative branches allocated 85% of the total dry weight to the terminal shoot and 15% to the lateral shoots.

4. The increase in total dry weight on reproductive branch units was not simply the result of a relocation of assimilates from the needles of the parent shoot (i.e. previous year's shoot). Although reproductive branch units had significantly less weight per needle

on the parent shoot the reduction was not as large as the increase in dry weight found on reproductive branch units. An estimated 23% more dry weight was present on reproductive branch units.

5. The results from this work suggest that the increase in total dry weight on reproductive branch units resulted from a combination of (i) a higher photosynthetic rate in the needles associated with male cones, (ii) importation of additional assimilates into the male cone-bearing branches, (iii) refixation of a significant proportion of the cones own respiratory CO_2 and possibly (iv) reduced exportation of assimilates from reproductive branch units. These suggestions are followed up in later chapters.

2.4 INFLUENCE OF FEMALE CONES

Determining the influence of female cones on vegetative growth of P. contorta was complex because it was very difficult to adequately compare: (i) between trees, because of genetic differences, (ii) within a tree, because of the pronounced gradients of vigour and associated sexual zonation, or (iii) between prolific and non-prolific coning years, because of environmental variables. The problems listed above were overcome when determining the influence of male cones on vegetative growth by comparing 'paired' vegetative and male cone-bearing branch units from the same position within the trees (section 2.3). However very few similarly 'paired' vegetative and female cone-bearing branch units could be found. Therefore in this study the variables known to primarily influence the current year's growth and development were taken as normalising or standardising variables and by fitting a stepwise regression model, which included the number and weight of female cones, it was possible to determine if female cones significantly influenced the growth and development of P. contorta branches.

2.4.1 MATERIALS

Branch units were removed at the base of the parent shoot. They consisted of: (i) a parent shoot which elongated in 1986, (ii) current year's shoots (terminal and laterals) which elongated in

1987, (iii) latent buds, and in some cases, (iv) female cones initiated in 1985, termed n2 cones (i.e. two-year-old) and those initiated in 1986, termed n1 cones (Figure 2.9). The n2 female cones were included in the current year's growth as this was the year of maximum dry weight increment (Dickmann & Kozlowski 1969). The n2 female cones were just about to shed their seed when sampled. Branch units which had scars, indicating that female cones had aborted, were excluded from the study. Latent buds were initiated at the same time as lateral shoots but do not differentiate needles or grow the following spring. They were recognisable as small buds amongst the lateral shoots (Figure 2.9). The number of lateral shoots and latent buds combined together give the number of potential shoots a branch unit might have had the following year. The total number of lateral shoots + latent buds will be termed the 'potential number of lateral buds' on a branch unit.

One hundred and six branch units were collected between the 15th September and 10th October 1987, from five individual trees growing at the Bush Estate, Penicuik, Scotland. Trees 1-3 were 20 years old, while trees 4 and 5 were 16 years old. These trees were chosen as they had both vegetative and reproductive branch units in similar positions in the tree crown. The vertical distribution of reproductive and vegetative branch units is shown in Figure 2.10 for trees 1-3. The position of the branch unit was recorded by counting the number of whorls from the top of the tree. Trees 4 and 5 had been sampled too often in the past to accurately assess the whorls. The sample consisted of 47 branch units with n2 female cones attached, which will be termed reproductive branch units, and 59 branch units without n2 female cones which will be termed vegetative branch units (Table 2.9). It was not possible to restrict the sampling of vegetative branch units to branch units without n1 female cones because of the lack of suitable material. However as the n1 female cones weighed less than 0.6g (about 1% of the total dry weight of the current year's growth), it is unlikely that they greatly influenced the parameters investigated in this study.

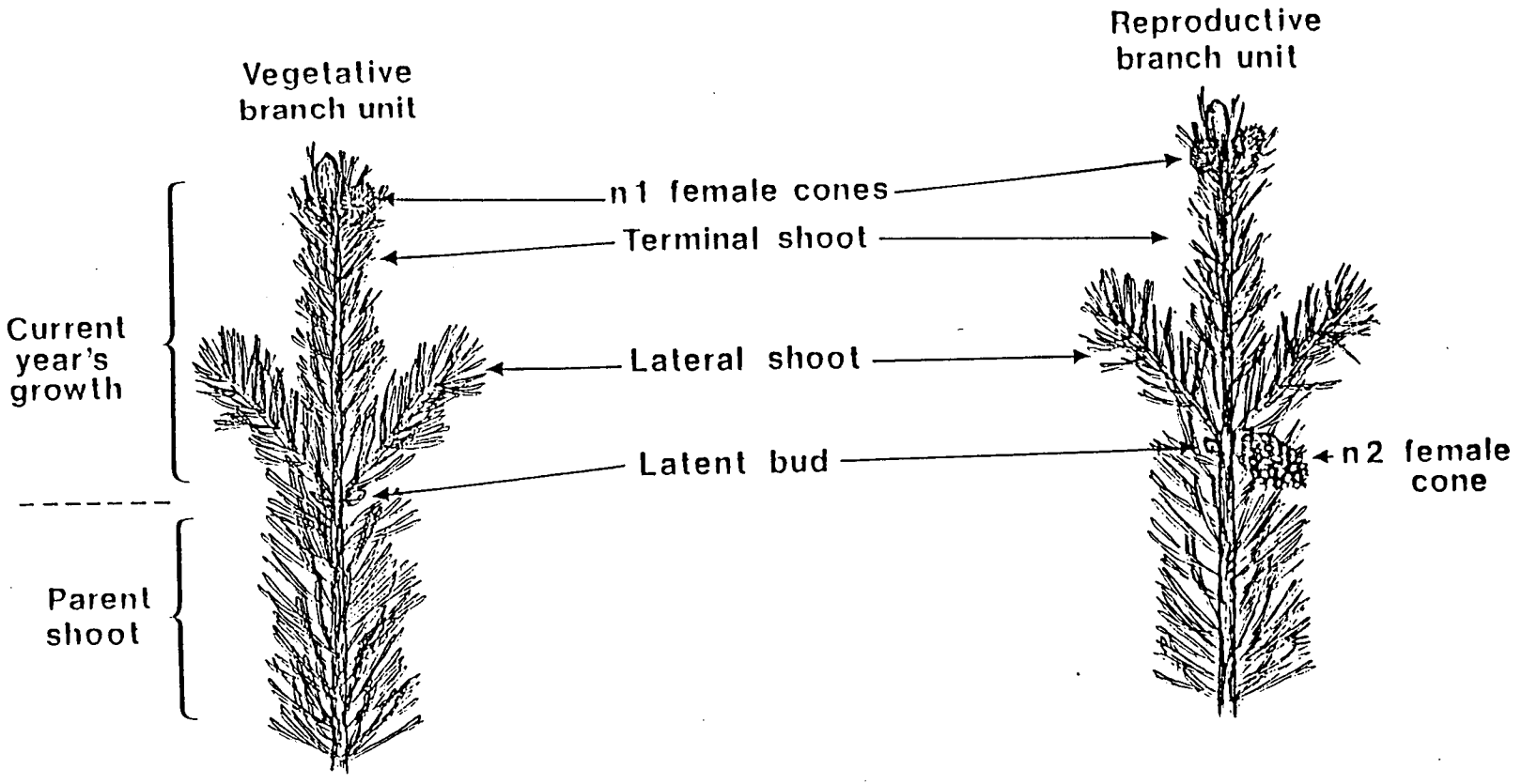


Figure 2.9 Diagrammatic representation of vegetative and female cone-bearing branch units sampled from P. contorta trees.

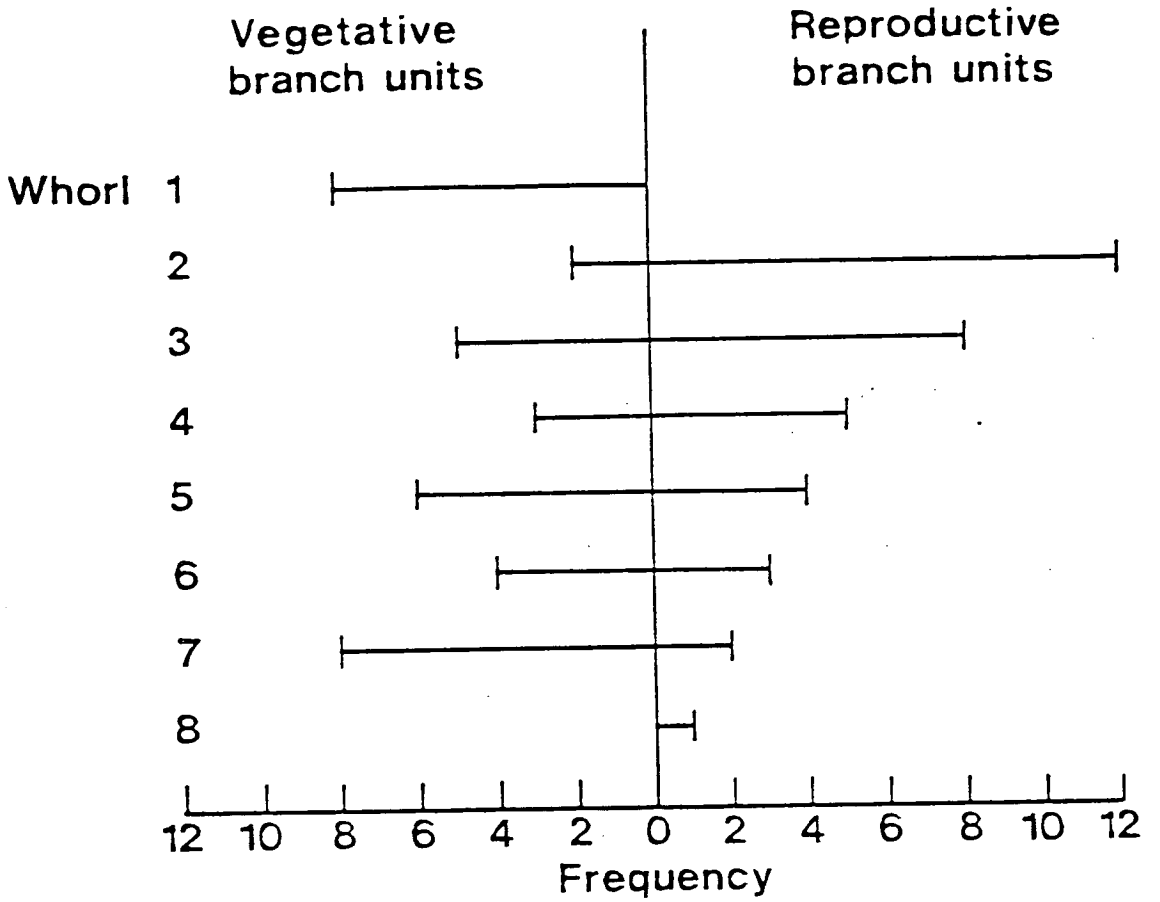


Figure 2.10 Frequency of vegetative and female cone-bearing branch units sampled at eight positions on three trees of P. contorta.

Table 2.9 Number of parent, terminal and lateral shoots on vegetative and reproductive branch units sampled from five P. contorta trees.

Tree	Type of branch unit	NUMBER OF SHOOTS Parent	Terminal	Lateral
1	Reproductive	11	11	33
	Vegetative	8	8	26
2	Reproductive	14	14	53
	Vegetative	18	18	63
3	Reproductive	10	10	52
	Vegetative	9	9	42
4	Reproductive	8	8	24
	Vegetative	17	17	42
5	Reproductive	4	4	9
	Vegetative	7	7	21
TOTAL	Reproductive	47	47	171
	Vegetative	59	59	194
GRAND TOTAL		106	106	365

2.4.2 METHODS

Branch units were cut and taken to the laboratory, where they were stored at 4 °C until they were assessed, usually the same day. The following variables were recorded in each branch unit:

(i) current year's growth on both terminal and lateral shoots;
(a) length, diameter and dry weight of the stem,
(b) number and dry weight of n1 female cones,
(c) number and dry weight of lateral plus latent buds,

(ii) parent shoots;
(a) length, diameter and dry weight of the stem,
(b) number and dry weight of n2 female cones,
(c) number and dry weight of lateral shoots,
(d) number and dry weight of latent buds and
(e) the number, area and dry weight of needle pairs.

Dry weights were determined by placing all parent, terminal and lateral shoots in separate bags and drying at 80 °C to constant weight.

A stepwise regression model was utilised to determine which of the recorded variables were significantly associated with the:

(i) number of potential lateral buds,
(ii) number of lateral shoots,
(iii) number of latent buds,
(iv) length of the terminal shoot,
(v) total length of all the lateral shoots,
(vi) total dry weight of the current year's growth,
(vii) dry weight of the terminal shoot and
(viii) dry weight of the lateral shoots.

Only variables which occured at the same time could be fitted in these regression models. For example, in the model for the number of lateral shoots which were initiated in summer 1986, the dry weight of the parent stem could not be fitted as a variable because it was not weighed until the autumn of 1987. The genotype (i.e. tree number) was fitted in each model this indicates genetic differences between trees.

When analysing counts (e.g. number of lateral shoots) the data were transformed (log n+1) and the error assumed to have a Poisson distribution, for continuous variables (e.g. dry weight of the terminal shoot), the data were untransformed and a normal error distribution was assumed.

2.4.3 RESULTS

Number of n1 female cones

Significantly more reproductive branch units, produced n1 female cones than did vegetative branch units (Table 2.10). Only 13% of reproductive branch units did not have n1 female cones while 42% of vegetative branch units were entirely vegetative.

Table 2.10 Frequency of vegetative and reproductive branch units of P. contorta with n1 female cones.

Branch unit	BRANCH UNITS WITH n1 Female cones present	n1 Female cones absent	Total
Reproductive	41	6	47
Vegetative	34	25	59

X^2 with 1 degree of freedom = 11.082, $P<0.001$

Number of potential lateral buds, lateral shoots and latent buds

On the parent shoot, the number of potential lateral buds (i.e. lateral shoots plus latent buds) was significantly associated with number of needles on the parent shoot and the number of n2 female cones (Table 2.11). The coefficient from the regression analysis was used to determine the reduction in the number of potential lateral buds on branch units with one to four n2 female cones (Table 2.12). Genotype was not significantly associated with the number of potential lateral buds (i.e. there was no difference between trees in the number of potential lateral buds). This was the only model which did not show genetic differences.

The two components which comprise the potential number of lateral buds (number of lateral shoots and the number of latent buds), appear to be influenced by different factors. The number of lateral shoots was significantly associated with the number of needles on the parent shoot but not significantly associated with the number of n2 female cones (Table 2.11). In contrast the number of latent buds on the parent shoot was not significantly related to the number of needles on the parent shoot but was significantly associated with the number of n2 female cones (Table 2.11). The regression coefficients for the number of n2 female cones were negative indicating that the number of latent buds was reduced on branches with n2 female cones.

Table 2.11 Analysis of deviance for three stepwise regressions to determine the influence of parent shoot and n2 female cone variables on: (i) the number of potential lateral buds, (ii) the number of lateral shoots and (iii) the number of latent buds of P. contorta (DF = degree of freedom, sign of the regression coefficient in brackets).

(i) Potential lateral buds

	DF	Deviance	Mean Deviance	Mean deviance ratio	P value	
No. needle pairs	1	17.7987	17.7987	86.81	< 0.001	(+)
No. n2 female cones	4	4.2156	1.0539	5.14	< 0.01	(-)
Genotype	4	1.0456	0.2614	1.27	ns	
Residual	96	19.6828	0.2050			

(ii) Lateral shoots

	DF	Deviance	Mean deviance	Mean deviance ratio	P value	
No. needle pairs	1	22.2648	22.2648	113.71	< 0.001	(+)
Genotype	4	2.8467	0.7117	3.63	< 0.01	
No. n2 female cones	4	0.5416	0.1354	0.69	ns	
Residual	96	18.7976	0.1958			

(iii) Latent buds

	DF	Deviance	Mean deviance	Mean deviance ratio	P value	
Genotype	4	10.5918	2.6480	3.66	< 0.01	
No. n2 female cones	4	25.2301	6.3075	8.71	< 0.001	(-)
No. needle pairs	1	0.0005	0.0005	0.00	ns	
Residual	96	69.5209	0.7242			

Table 2.12 The estimated reduction in the number of potential lateral buds on branches with n2 female cones of P. contorta.

Number of n2 female cones	Estimated reduction in the number of potential lateral buds
1	8 %
2	11 %
3	17 %
4	29 %

It appears that n2 female cones reduce the number of potential lateral buds but do not reduce the number of lateral shoots (Figure 2.11a). Female cones, therefore, are initiated at the expense of latent buds. However, it is not a simple replacement of one female cone for one latent bud, reproductive branch units had a larger total number of lateral shoots plus latent buds plus n2 female cones than did vegetative branch units (Figure 2.11b).

Length of terminal and lateral shoots on current year's growth

The length of the terminal shoot was significantly associated with the length of the parent shoot and the area per needle on the parent shoot, both the regression coefficients were positive indicating that branch units with the longest parent shoot and the greatest area per needle on the parent shoot would have the longest terminal shoot. The length of the terminal shoot was not significantly influenced by either the number or the weight of n2 female cones, or the number of lateral shoots (Table 2.13). The total length of lateral shoots was significantly associated with; the number of lateral shoots, the area per needle of the parent shoot and the total weight of the n2 female cones (Table 2.13). The regression coefficients were all positive, indicating that branch units with the greatest number of lateral shoot, the largest area per needle on the parent shoot and the greatest weight of n2 female cones had the greatest total length of lateral shoots. Therefore the length of the terminal and lateral shoots were not reduced by the presence of n2 female cones. In fact the weight of n2 female cones were found to be positively associated with the length of the lateral shoots.

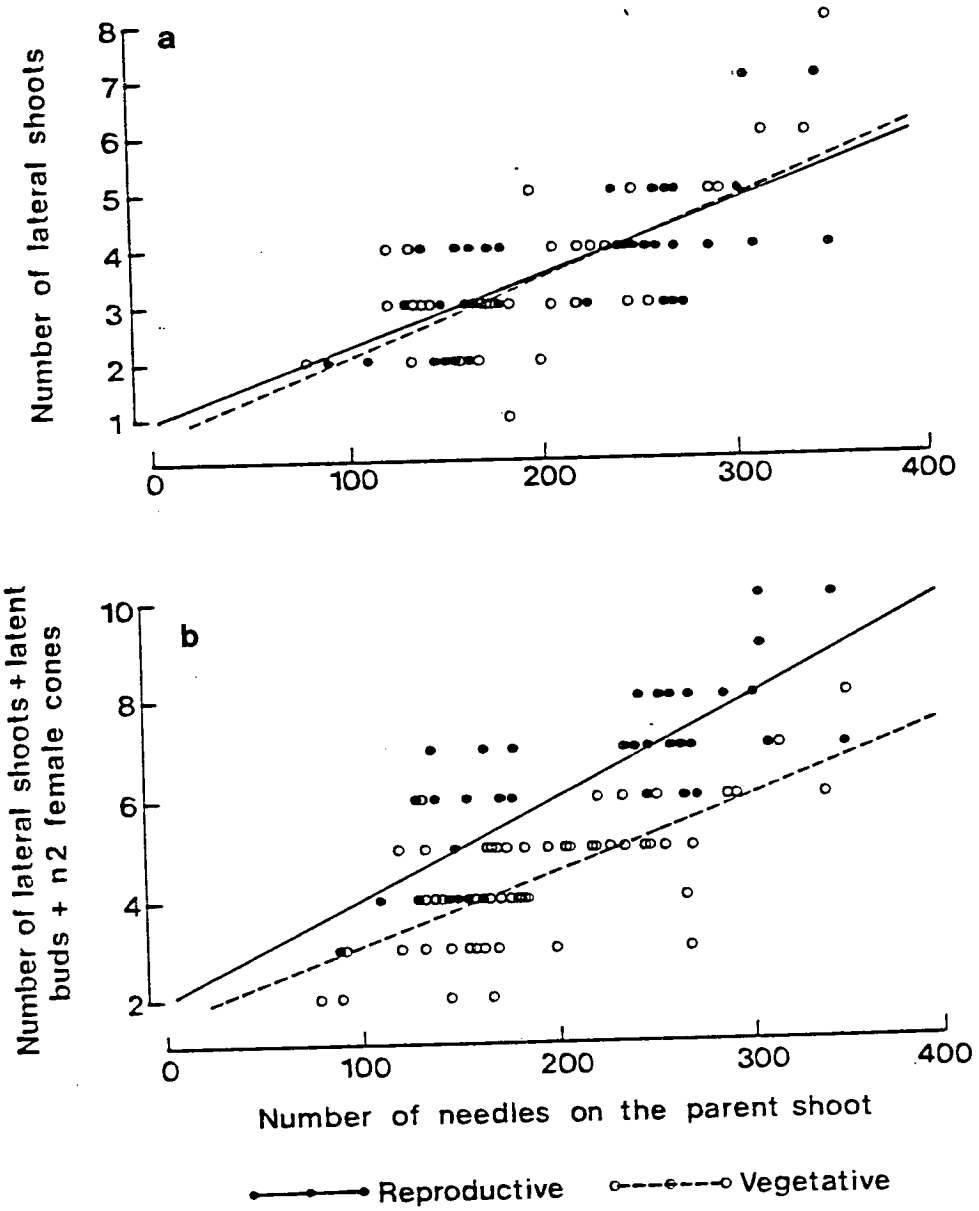


Figure 2.11 Relationship between a) the total number of lateral shoots, and b) the total number of lateral shoots plus latent buds plus n2 female cones with the number of needles on the parent shoot of vegetative and reproductive branch units of P. contorta.

Table 2.13 Analysis of variance for two stepwise regressions to determine the influence of parent shoot and n2 female cone variables on the lengths of: (i) the terminal and (ii) the lateral shoots of P. contorta (sign of the regression coefficient in brackets).

(i) Length of terminal shoot

	DF	SS	MS	F	P
Genotype	4	335024	83756	54.58	< 0.001
Length	1	48369	48369	31.52	< 0.001 (+)
Area per needle	1	24643	24643	16.06	< 0.001 (+)
No. lateral shoots	7	21712	3102	2.02	ns
No. n2 cones	4	10820	27054	1.76	ns
No. needles	1	5463	5463	3.56	ns
Weight of n2 cones	1	2039	2039	1.33	ns
Residual	86	131980	1535		

(ii) Total length of all lateral shoots

	DF	SS	MS	F	P
No. lateral shoots	7	4814107	687730	80.92	< 0.001 (+)
Genotype	4	1492965	373241	43.92	< 0.001
Area per needle	1	268867	268867	31.64	< 0.001 (+)
Weight of n2 cones	1	161877	161877	19.05	< 0.001 (+)
No. n2 cones	4	49792	12448	1.46	ns
No. needles	1	19523	19523	2.30	ns
Length	1	1276	1276	0.15	ns
Residual	86	730866	8498		

Dry weight allocation

The total dry weight of all the current year's growth was significantly associated with: the stem volume and density of the parent shoots; the number and dry weight per needle on the parent shoot; and the type of branch unit (Table 2.14). All the regression coefficients were positive indicating that reproductive branch units had significantly more total dry weight in the current year than equivalent vegetative branch units.

The total dry weight of the terminal shoots was significantly associated with: the stem volume and density of the parent shoot; the dry weight per needle on the parent shoot; and the dry weight of

the lateral shoot (Table 2.14). Neither the weight of n2 female cones nor the number of needles nor the area per needle on the parent shoot significantly influenced the total dry weight of the terminal shoot.

Table 2.14 Analysis of variance tables of three stepwise regressions to determine the influence of parent shoot and n2 female cone variables on: (i) total dry weight produce in the current year, (ii) dry weight of the terminal shoot and (iii) total dry weight of all the lateral shoots on branch units of P. contorta (sign of the regression coefficient in brackets).

(i) Total dry weight of all current growth ie including cones

	DF	SS	MS	F	P	
Stem volume	1	24691	24691	788.85	< 0.001	(+)
Type of branch unit	1	2696	2696	86.16	< 0.001	(+)
Stem density	1	1392	1392	44.47	< 0.001	(+)
Genotype	4	1844	461	14.73	< 0.001	(+)
Dry weight per needle	1	403	403	12.89	< 0.001	(+)
No. needles	1	104	104	3.34	ns	
Area per needle	1	1	1	0.02	ns	
Residual	95	2973	31			

(ii) Total dry weight of the terminal shoot

	DF	SS	MS	F	P	
Stem volume	1	3264	3264	488.11	< 0.001	(+)
Dry weight of laterals	1	323	323	48.32	< 0.001	(+)
Stem density	1	119	119	17.88	< 0.001	(+)
Genotype	4	256	64	9.56	< 0.001	
Dry weight per needle	1	87	87	13.08	< 0.001	(+)
Area per needle	1	24	24	3.54	ns	
Dry weight of n2 cones	1	10	10	1.55	ns	
No. needles	1	4	4	0.58	ns	
Residual	94	629	7			

(iii) Total dry weight of all lateral shoots

	DF	SS	MS	F	P	
Dry weight of terminal	1	5754	5754	349.65	< 0.001	(+)
Stem volume	1	418	418	25.40	< 0.001	(+)
Stem density	1	130	130	7.89	< 0.01	(+)
Genotype	4	304	76	4.61	< 0.05	
Area per needle	1	123	123	7.48	< 0.01	(+)
No. needles	1	84	84	5.13	< 0.05	(+)
Dry weight per needle	1	8	8	0.48	ns	
Dry weight of n2 cones	1	4	4	0.24	ns	
Residual	94	1547	16			

The total dry weight of all the lateral shoots was significantly associated with: the dry weight of the terminal shoot; the stem volume and density of the parent shoot; the number of needles and the area per needle on the parent shoot (Table 2.14). Neither the dry weight of the n2 female cones nor the dry weight per needle on the parent shoot were significantly associated with the total dry weight of the lateral shoots.

Therefore although reproductive branch units had more total dry weight on the current year's growth compared to vegetative branch units, n2 female cones did not reduce the dry weight allocated to the terminal or lateral shoots.

The allocation of dry weight between the stem, needles and buds was in the same proportions on reproductive branch units and on vegetative branch units of all trees studied (Figure 2.12a). Between 17% and 45% of the total dry weight in the current year was allocated to n2 female cones, while only 1% to 5% was allocated to n1 female cones.

2.4.4 DISCUSSION

This study shows that, contrary to published opinion (Ebell 1971, Cannell 1976, 1979), the presence female cones does not result in fewer lateral shoots on a branch in P. contorta, although the number of latent buds was reduced. The latent buds may be present as an 'insurance strategy' for the tree. Therefore, female cones could be considered as limiting the potential, although not the actual growth of shoots.

Female cones, in fact, were positively associated with the total length of all the lateral shoots. This positive association implies that the reproductive branch units actually grew more per unit of parent shoot compared with vegetative branch units. This increased growth may have resulted because n2 female cones, by creating a larger sink, increased the photosynthetic rate of the needles on the parent and current year's shoots (see review by Neales & Incoll 1968, Bidwell 1983, Weaver & Johnson 1985). Little evidence to

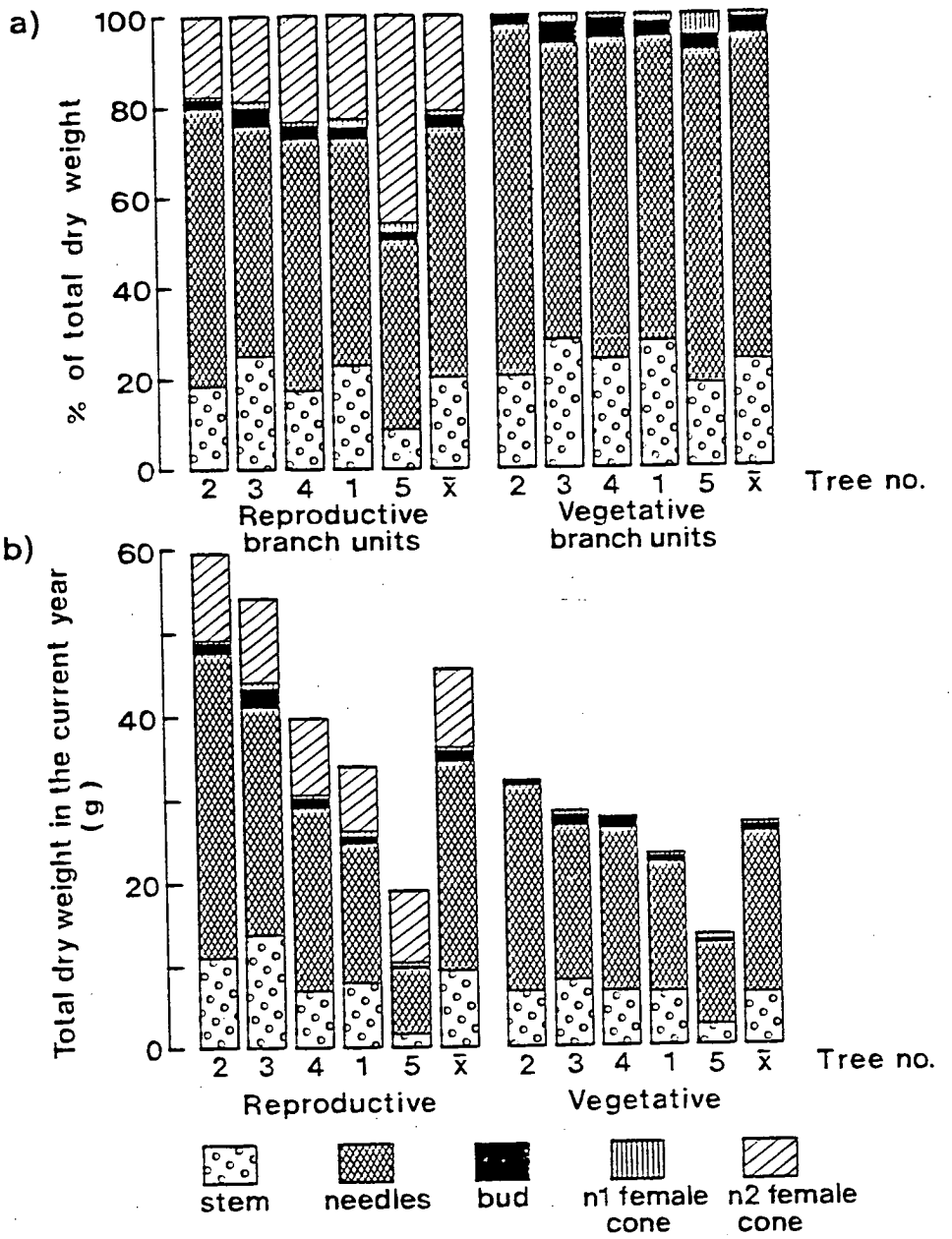


Figure 2.12 Variation in a) the percentage and b) the total dry weight of the current year's growth of vegetative and reproductive branch units of P. contorta.

support this hypothesis was found when the gas exchange of needles on the parent shoots of reproductive branch units was compared with closely associated vegetative branch units (Chapter 3). However it is possible, that female cones induce a higher photosynthetic rate on the surrounding vegetative branches as well as the branch units which bear them. This is a resonable hypothesis because female cones have been shown to have a higher competitive ability for assimilates than vegetative structures (Wardlaw 1968, Dickmann & Kozlowski 1968) and to attract assimilates from surrounding branches (Dickmann & Kozlowski 1968). It would appear, therefore, that increased importation of assimilates, from surrounding branches, led to the increased growth and dry weight of reproductive branch units. Female cones also contribute to their own carbon economy by reducing the loss of respired CO2 by reassimilation during the day (Dickmann and Kozlowski 1970, Linder & Troeng 1981).

In this study, reproductive branch units did not have reduced vegetative growth or reduced dry weight by the end of the year, despite an allocation of between 17% - 45% of the total current year dry weight to n2 female cones. Since both reproductive and vegetative branch units were on the same trees it was not possible to determine the influence of female cones on other aspects of whole tree growth. There may not, however, be much direct competition for assimilates between the female cones and the roots and stem of the tree. This hypothesis is supported in the literature because it has been shown that most assimilates are allocated to the roots and stem after the growth of the current shoot has ceased (Shiroya et al. 1966, Ursino et al. 1968, Schier 1970) and this is also after the n2 female cone have ceased to grow (Dickmann & Kozlowski 1969, Linder & Troeng 1981). It would appear therefore that when the tree is not stressed, there is little evidence of direct competition between female cones and stem wood. It may not be valid, therefore, to assume that reproductive structures are produced at the expense of a similar weight of stem wood (Fielding 1960, Ovington 1963). However, it is possible, if assimilates are limited, that female cones may reduce the root extension and stem increment of trees because radioactive labelling studies (see Ericsson 1980) have shown that the roots and the stem are lower priority sinks compared with

the current years growth including the female cones. This requires further study.

As an alternative hypothesis, the reduction in growth so often associated with female cones in trees may be the result of adverse weather conditions the previous year. Hot dry weather, for example, is known to enhance the development of sexual structures in many plants (see reviews by Lee 1979, Owens & Blake 1985) but also to reduce the number of needle pairs formed (Cannell 1976). As these needles develop in the same year as the n2 female cones and are the major source of assimilates for the growth of the tree in that year, the reduction in tree growth may be the result of a lower needle area rather than the presence of female cones.

In conclusion it would appear from this study that female cones do not adversely influence vegetative growth during a normal year, although the possibility cannot be excluded that if the tree was stressed in some way, for example by drought, lack of nutrients, pollution etc., the situation might be different.

2.4.5 SUMMARY

1) The number of lateral shoots which differentiated and developed needles was not reduced on branch units with n2 female cones. However, the number of latent buds was significantly reduced by the presence of n2 female cones.

2) The length of the terminal shoot was not associated with either the weight or number of n2 female cones. Whereas, the total length of all lateral shoots was positively associated with the weight of n2 female cones.

3) Branch units with n2 female cones produced significantly more total dry weight in the current year (i.e. terminal + lateral shoots + n1 + n2 female cones). There was however, no significant reduction in the vegetative growth (i.e. terminal + lateral shoots).

4) Therefore female cones apparently do not reduce the vegetative growth of the branches which bear them. It was suggested that female cones may result in: (i) an increase in the photosynthetic efficiency of associated needles, (ii) enhanced importation or reduced exportation of assimilates from other parts of the tree not assessed in this study, and (iii) they produce assimilates by refixation of respiratory CO_2.

2.5 COMPARING THE INFLUENCE OF MALE AND FEMALE CONES ON SHOOT GROWTH

Male and female cones apparently influence the growth of P. contorta trees in different ways. Male cones significantly reduce the number of needles and lateral shoots on branches which bear them, while female cones do not reduce either, although female cones do result in less latent buds on shoots bearing female cones.

Reproductive branches, either male or female, have a larger dry weight than equivalent vegetative branches when the cones are fully developed. The source of the increased dry matter may be slightly different for the two sexes of cones. Male cones have been found to increase the photosynthetic rate of the needles immediately adjacent to them, i.e. the needles on the parent shoot, while no such increase has been found on branches which bear female cones (Chapter 3). It is suggested that female cones may induce a higher photosynthetic rate on other associated branches as well as on the parent branch and import assimilates form a wider area than male cones. Male cones are only present on the tree for 10 to 12 months compared to female cones which take 28 to 30 months to mature. This difference in phenology might account for female cones having more influence on their surrounding branches. Both male and female cones are also known to have a reduced CO2 loss if exposed to light (Chapter 5), thereby contributing to their own carbon economy.

In conclusion, male cones reduce both the number of needles and lateral shoots on the branches which bear them, while female cones have little, if any, detrimental effect on the shoot growth of P. contorta trees.

CHAPTER 3

INFLUENCE OF MALE AND FEMALE CONES ON RATES OF NEEDLE PHOTOSYNTHESIS AND RESPIRATION IN THE FIELD

3.1 AIM

Reproductive structures of many plants have been found to enhance the photosynthetic rate of the leaves immediately adjacent to them. It was the aim of this study to determine if male and female cones induce a higher rate of CO_2 exchange on the branches which bear them as compared with equivalent vegetative branches.

3.2 INTRODUCTION

Both male and female cone-bearing branches have more total dry weight when the cone is fully developed, compared to equivalent vegetative branches (Chapter 2). One possibility is that the increase in dry weight results from an increase in the rates of photosynthesis of the needles most closely associated with the reproductive structures. Reproductive structures are a major sink for assimilates in many plants (Kriedemann 1969, Hansen 1977, Hofstra 1984, Ho 1988) including conifers (Wardlaw 1968, Dickmann & Kozlowski 1970b). Although a direct causal relationship between sink activity and the photosynthetic rate of leaves has not been proven, there are many examples of leaves close to reproductive structures having increased assimilation rates (see reviews by Neales & Incoll 1968, Evans 1968, Gifford & Evans 1981). There are, however, also examples of reproductive structures that have not apparently altered the CO_2 exchange rate of plants, particularly toward the end of the growing season (Hansen 1970). It was the aim of this study to determine if male and female cones induced a higher rate of CO_2 exchange in the branches which bore them as compared with equivalent vegetative branches.

As male and female cones have separate periods of growth, the CO_2 exchange rate of associated needles was measured throughout the growing season in the field. Needles on the previous year's shoot

were examined on three branch types (vegetative, male and female cone-bearing). The needles on the previous year's shoot, the so-called 'parent shoot' (Cannell 1976), were chosen as they are known to be the major source of assimilates for the current year's growth, including the cones (Clausen & Kozlowski 1967, Dickmann & Kozlowski 1968, Gordon & Larson 1968, Loach & Little 1973, Chung & Barnes 1980, Ericsson 1980).

Since the environmental variables which influence assimilation rate varied throughout the measurement period, a theoretical model was fitted to the data. There are several published models that relate assimilation rate to environmental variables such as quantum flux density, temperature and CO_2 concentration (see Miranda 1982). The model published by Jarvis et al. (1985) was utilised in this study, because it relates leaf assimilation to the environmental and physiological variables that seem to be important. The model essentially fits a non-rectangular hyperbola to the assimilation (A) and quantum flux density data (Q), while also estimating the resistance of CO_2 transfer into the carboxylation sites.

3.3 MATERIALS

In March 1985 eight clonal cuttings were chosen from an unshaded south-facing bank of trees which were planted in 1972 at the nursery of the Forestry Commission's Northern Research Station, Bush Estate, near Edinburgh. The cuttings (clone number 8020) had been collected and rooted in 1971 from an inter-provenance hybrid (Queen Charlotte Island x Olympic) growing at Broxa Forest, Yorkshire, England.

From these eight trees a total of 30 branches were selected. Ten each with vegetative buds, male-cone buds and two-year-old female cones (n2). These will be termed vegetative, male cone-bearing and female cone-bearing branches, respectively, throughout this chapter. The branches were carefully selected such that, wherever possible, they came from the same branch whorl, with similar aspect and length of parent shoot (Table 3.1). Thus similar branches were 'grouped', one of each type from the same crown position. This was because it has been shown (Lewandowska 1976, Rook & Corson 1978,

Troeng & Linder 1982b) that needles of the same age class have different photosynthetic rates depending on their positions within the tree crown.

Table 3.1 Summary of morphological variables of vegetative, male and female cone-bearing branches of P. contorta (mean of ten branches with one standard error of the mean in brackets).

	BRANCH TYPE		
	Vegetative	Female	Male
Length of parent shoot (mm)	98.2 (5.9)	113.9 (7.6)	96.7 (3.5)
Length of terminal bud (mm)	25.5 (1.3)	31.6 (2.3)	30.8 (1.4)
Diameter of parent shoot (mm)	0.5 (0.02)	0.6 (0.01)	0.5 (0.03)
Number of :-			
1st order branches	0	0	1
2nd order branches	8	12	8
3rd order branches	4	0	3
Female cones per shoot	0	1.5	0
Male cones per shoot	0	0	41

Some needles were removed from the parent shoot to allow a leaf chamber to fit around the shoot. The plan area of the needles removed was measured using a leaf area meter (Model 3100, LiCor Inc., Lincoln, Nebraska, USA). The area per needle was calculated and used to estimate the area of the needles enclosed in the chamber (Table 3.2).

Table 3.2 Mean number and area of needle pairs removed, sampled or left on the shoot (but not enclosed within the leaf chamber) of vegetative, male and female cone-bearing branches of P. contorta (one standard error of the mean in brackets).

	BRANCH TYPE		
	Vegetative	Female	Male
Number of needle pairs			
Removed	37 (3.3)	38 (2.5)	33 (2.8)
Outside the chamber	22 (3.4)	25 (3.1)	20 (4.1)
Sampled in chamber			
in May	52 (2.6)	44 (3.8)	54 (6.9)
in July	55 (3.0)	39 (4.0)	50 (6.3)
Area of needle pairs (cm^2)			
Removed	35.2 (3.2)	39.0 (3.3)	28.1 (2.6)
Outside chamber	20.8 (2.8)	31.7 (7.1)	17.0 (3.6)
Sampled in chamber			
in May	48.0 (2.1)	43.9 (3.2)	44.5 (5.4)
in July	44.4 (2.6)	39.1 (3.8)	41.2 (4.8)

3.4 METHODS

The null-balance system described by Griffiths and Jarvis (1981) was used to measure the uptake or release of carbon dioxide from the needles within the leaf chamber. This system essentially bleeds in CO_2 from a cylinder, until the flow rate exactly balances the uptake or release of CO_2 from the needles (Figure 3.1). An infra-red gas analyser (Binos 1, Leybold-Heraeus GMBH, Hanau, Germany), in absolute mode, was used to measure the concentration of CO_2 within the chamber. The flow rate from the cylinder was adjusted so that the concentration of CO_2 in the chamber was 340 $\mu mol\ mol^{-1}$. Two CO_2 cylinders were used, either 100-150 $\mu mol\ mol^{-1}$ more or less than the balancing concentration in the chamber, depending on whether the needles were assimilating or respiring CO_2.

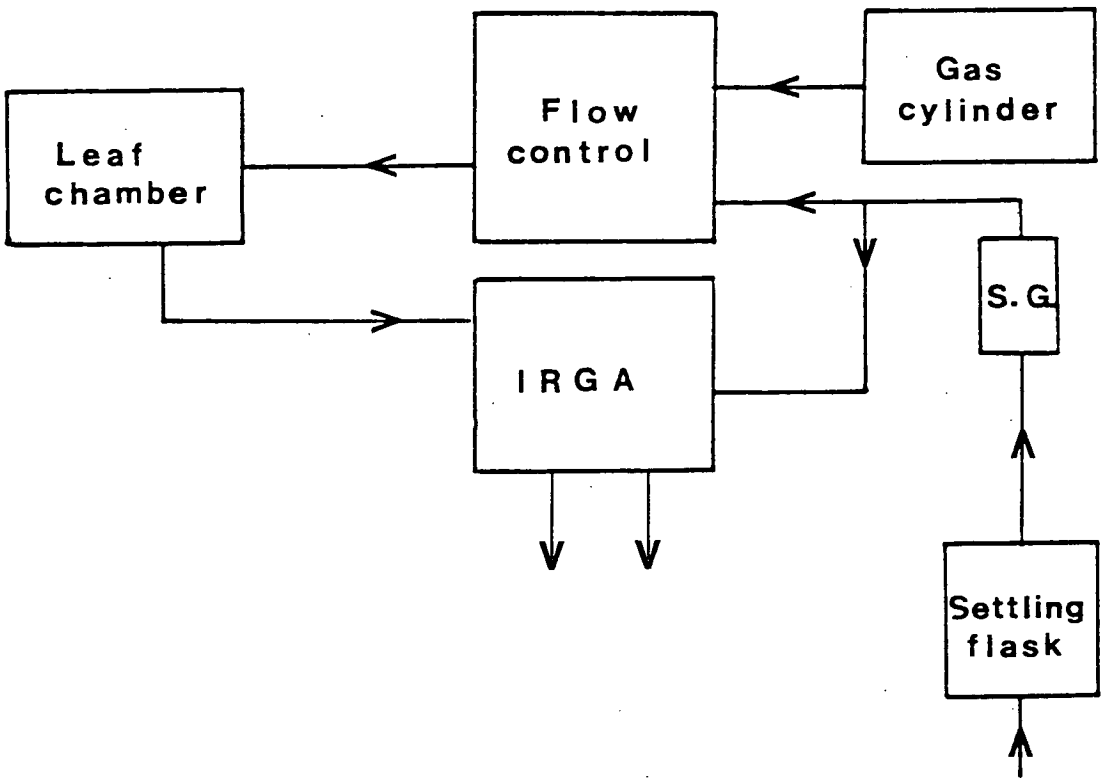


Figure 3.1 Diagram of the null balance system used to measure the uptake and release of carbon dioxide from needles on parent shoots of reproductive and vegetative branches of P. contorta (adapted from Griffiths and Jarvis 1981, S.G. = silica gel).

A leaf chamber was especially designed and built for this study (Figure 3.2). The walls were constructed of acrylic plastic, glued together with 'Tensol' cement NO. 70 a methyl methacrylate mixture (20:1, A:B). The inside of the chamber was lined with Teflon FEP film (type 500 C, gauge 0.13 mm, Du Pont de Nemours International, Geneva, Switzerland) which has low CO_2 and H_2O absorbing properties. The lining was attached with double-sided Sellotape. The chamber was held closed with adjustable clips (Protex Fastners Ltd, Worcestershire, England) and rubber foam strips were stuck on to the edge (Grade 515, Silicone Engineering, Blackburn, Lancashire, England).

To ensure a homogenous gaseous mix, a 12 volt DC servo motor (Farnell Electronic Components Ltd, Canal Road, Leeds, England), was positioned in the bottom of the chamber and turned a teflon plug fitted with an aluminium blade. The cooling curve technique of Grace et al. (1980), was used to determine the boundary layer conductance of brass needle replicas within the chamber. A boundary layer conductance of 0.114 m s^{-1} was used in subsequent calculations.

An air temperature sensor (AD590, RS Components, London, England) and humidity sensor (Humicap Vaisala (UK) Ltd, Cambridge, England) were placed in the air stream leaving the chamber. A quantum sensor (LI 190 LiCor Inc., Lincoln, Nebraska, USA) was fitted to the outside of the chamber in such a way that it could be moved to give the best estimate of the quantum flux density in the chamber.

The output from the sensors in the chamber were displayed on a null-balance H_2O porometer (Dingbat Electronics, Aberdeen, Scotland) as described by Griffths & Jarvis (1981) , which also housed the flow controller (P5 RDP Electronics Ltd., Wolverhampton, England).

All the instruments were calibrated in early May and again in early October 1985. The flow meter was calibrated against a Tylan mass-flow meter (Model FC261, Tylan Corporation, Surrey). To calibrate the humidity sensor, a water bath (Model Dp35, Grant Instruments (Cambridge) Ltd., Barrington, Cambridge, England) was

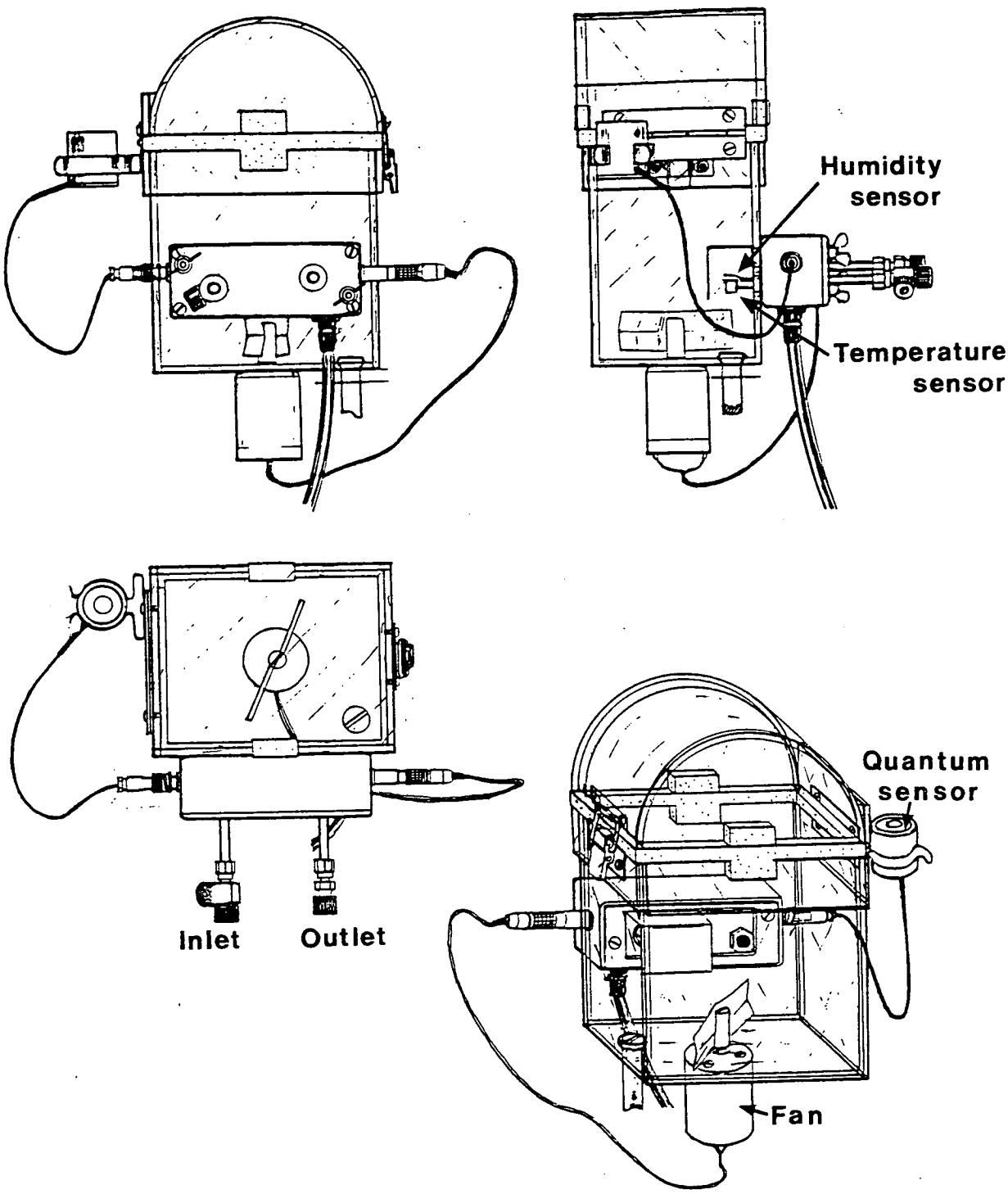


Figure 3.2 Diagram of the leaf chamber designed and used to measure the CO_2 exchange rate of needles on parent shoots of reproductive and vegetative branches of P. contorta.

used to generate water-saturated air by condensing out water from presaturated air in copper coils at temperatures between 3 °C and 20 °C. This gave a range of relative humidities between 26% and 85% at a chamber temperature of 23.0 °C. Zero humidity was achieved by blowing nitrogen gas from a cylinder into the chamber for 15 minutes. A platinum resistance thermometer (Model 9535, Guildline Instruments Ltd., Smiths Falls Ontario. Canada) was placed inside the chamber to calibrate the temperature sensor in the range of 6 °C to 30 °C. A recently purchased quantum sensor (LI 190, LiCor Inc., Lincoln, Nebraska, USA) was used to calibrate the chamber quantum sensor by placing both in direct sunlight and covering with neutral density filters (Frew-Smith Ltd., Irvine, Scotland). A set of three gas-mixing pumps (Models 1 SA 18/3F, 1 SA 2 AND 1 G 27/3F, H. Wosthoff, D463 Bochum, Germany) was used to generate carbon dioxide concentrations of 300 and 500 μmol mol^{-1}. These concentrations were used to calibrate the infra-red gas analyser. The zero point was obtained by using a tube of soda lime ('Carbosorb' BDH Ltd. Poole, England) to remove CO_2 from the air stream.

As the environmental conditions were constantly changing throughout the day, care was taken to ensure that each branch type was assessed under different environmental conditions. As the branches were 'grouped', one of each type (vegetative, male and female) the three branches within a 'group' were sampled randomly and the ten 'groups' were assessed sequentially throughout the day.

Photosynthesis and respiration data were not always collected on the same day because of rain showers (Table 3.3). On average the needles of the parent shoot were enclosed in the chamber for three minutes, when measuring the photosynthetic rate and five minutes, when measuring the respiration rate.

As 1985 was an extremely wet year in Scotland there were very few days throughout the growing season when the CO_2 exchange rate of the needles could be measured (Figure 3.3).

Table 3.3 Number of observations, maximum, mean and minimum temperature (temp., °C) vapour pressure difference (VPD, kPa) and stomatal conductance (g_s, mmol m^{-2} s^{-1}) calculated when measuring the: (i) respiration and (ii) photosynthetic rates of needles on the parent shoots of vegetative (V), male (M) and female (F) cone-bearing branches of P. contorta at each sampling time.

	SAMPLING PERIOD								
	Spring			Summer			Autumn		
(i) Respiration									
Dates	3-4/6/85			6/6/-10/7/85			15-26/10/85		
Number of Measuring days	2			7			7		
	V	M	F	V	M	F	V	M	F
Number of Measurements	24	24	24	37	37	37	58	58	58
Minimum temp.	17	17	17	12	12	12	10	10	10
Mean temp.	22	22	22	18	18	18	13	13	13
Maximum temp.	28	27	27	24	24	23	16	16	16
Minimum g_s	5	9	9	14	23	8	19	19	22
Mean g_s	13	15	11	73	85	68	54	65	60
Maximum g_s	60	52	34	255	250	200	153	173	176
Minimum VPD	1.2	1.3	1.3	0.1	0.1	0.1	0.1	0.1	0.1
Mean VPD	1.9	1.9	1.9	0.4	0.4	0.5	0.5	0.5	0.5
Maximum VPD	3.3	3.3	3.3	1.2	1.2	1.3	1.0	1.0	1.0
(ii) Photosynthesis									
Dates	23/5-3/6/85			10/6/-10/7/85			10-30/10/85		
Number of Measuring days	4			8			7		
	V	M	F	V	M	F	V	M	F
Number of Measurements	68	71	71	69	66	72	94	87	101
Minimum temp.	13	13	13	10	10	10	10	11	11
Mean temp.	19	19	19	18	18	18	15	15	15
Maximum temp.	27	29	29	27	28	28	20	20	20
Minimum g_s	7	39	24	10	13	4	12	34	37
Mean g_s	123	156	142	72	85	67	96	111	112
Maximum g_s	306	313	296	275	369	269	233	263	289
Minimum VPD	0.3	0.3	0.3	0.2	0.2	0.2	0.2	0.1	0.2
Mean VPD	0.7	0.7	0.7	0.6	0.5	0.6	0.4	0.4	0.5
Maximum VPD	1.3	1.2	1.5	1.3	1.4	1.7	0.8	0.8	0.8

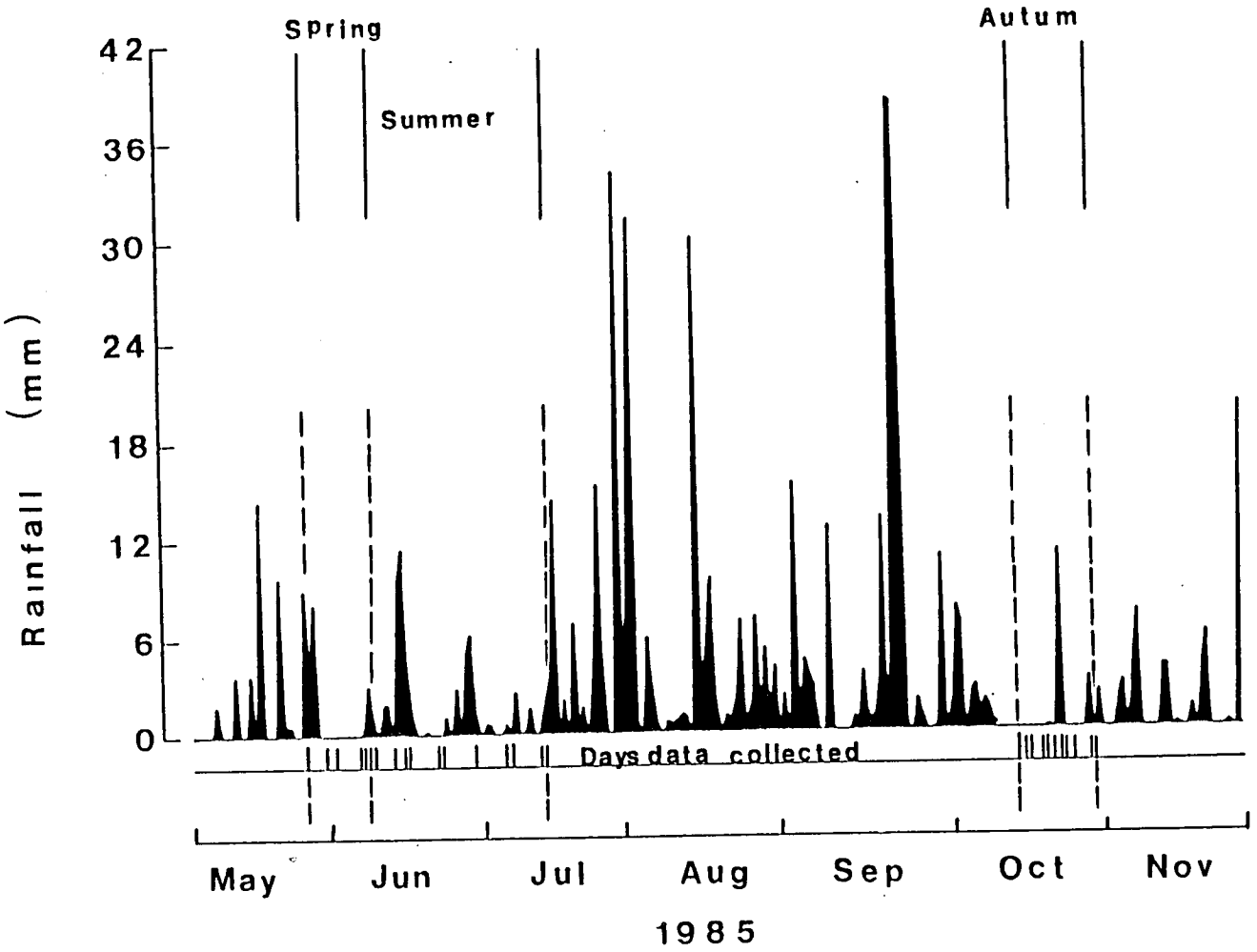


Figure 3.3 Daily rainfall from May to November 1985 at I.T.E., Bush Estate, Penicuik, approximately 100 m from the experimental site at the Forestry Commission's Northern Research Station.

The data from different dates were divided for analysis into three sets corresponding to three physiological stages in the life history of the cones:
(i) prior to pollen shed (23/5/85 to 4/6/85)- termed spring,
(ii) after pollen shed (6/6/85 to 10/7/85)- termed summer and
(iii) prior to seed shed (10/10/85 to 30/10/85)- termed autumn

Analysis of variance was used to test the significance of differences between the dark respiration rates of vegetative, male and female cone-bearing branches. The theoretical model of Jarvis et al. (1985), was fitted to the data for each branch type and each sample period. Quantum flux density (Q), stomatal conductance (g_s) and assimilation rate (A) are the measured input variables for the model. The optimising routine in a computer package (Genstat version 4) was used to estimate the parameter values with the least mean square error. The parameters estimated were:
(i) dark respiration (R_d),
(ii) mesophyll conductance (g_m),
(iii) the initial slope of the A/Q curve (α) and
(iv) the convexity coefficient (θ), which defines the degree of curvature between the initial slope and the asymptotic value of A.
The main assumptions in the model are:
(i) photosynthesis is related to quantum flux density by a non-rectangular hyperbola,

$$\theta(A+R_d)^2-(\alpha Q+A_{max}+R_d)(A+R_d)+\alpha Q(A_{max}+R_d)=0 \qquad (3.1)$$

(ii) photosynthesis is linearly related to C_i (internal concentration of carbon dioxide) over the range of interest,

$$A_{max} = (C_i - \Gamma)g_m \qquad (3.2)$$

A_{max} = maximum assimilation rate,
$\Gamma = CO_2$ compensation concentration, and
(iii) C_i depends on the rate of photosynthesis and the stomatal conductance,

$$C_i = C_a - (A/g_s). \qquad (3.3)$$

The estimated parameters were then used to plot A/Q curves using values for the mean stomatal conductance measured at each sample time for each branch type (Table 3.3). Also plotted on these graphs were both the measured and the predicted values of A using the

estimated parameters and the measured values of g_s. These plots (an expression of the residuals) were used to show the 'goodness of fit' of the model. A_{max} was calculated as shown in equation 3.3 where Γ was assumed to be 50 μmol mol^{-1} and the value of C_i was the mean value obtained for Q higher than 900 μmol m^{-2} s^{-1} in the spring and summer sample periods and higher than 600 μmol m^{-2} s^{-1} in the autumn sample period. The statistical difference between the A/Q curves were determined by a combined curve analysis of variance (Ross 1981), which tests the reduction in residual variance obtained by fitting a set of individual curves compared to the residual variance from a common curve. The predicted A/Q curves were drawn using the estimated parameters and a standard g_s value of 100 μmol m^{-2} s^{-1} for all branch types.

The length of the current year's terminal shoot was measured with a ruler from May to the end of June. The diameter of the n2 female cones was measured with calipers (Model CL1, Mackay and Lynn, Edinburgh).

3.5 RESULTS

3.5.1 GROWTH OF THE CURRENT SHOOT AND n2 FEMALE CONE

Extension of the current year's terminal shoot had ceased by the end of June 1985 on all three branch types (Figure 3.4). The increase in diameter of the n2 female cones also ceased around this time (Figure 3.5). Female cone-bearing shoots grew significantly longer than vegetative shoots, which in turn were significantly longer than male cone-bearing shoots (Figure 3.4).

3.5.2 RESPIRATION

Female cone-bearing branches had higher average rates of dark respiration than vegetative branches at all three sampling times. Female cone-bearing branches also had higher rates of dark respiration than male cone-bearing branches at both the spring and summer sampling times, but not in the autumn sampling period. There were no significant differences between the vegetative and male cone-bearing branches, at any of the sample periods (Figure 3.6).

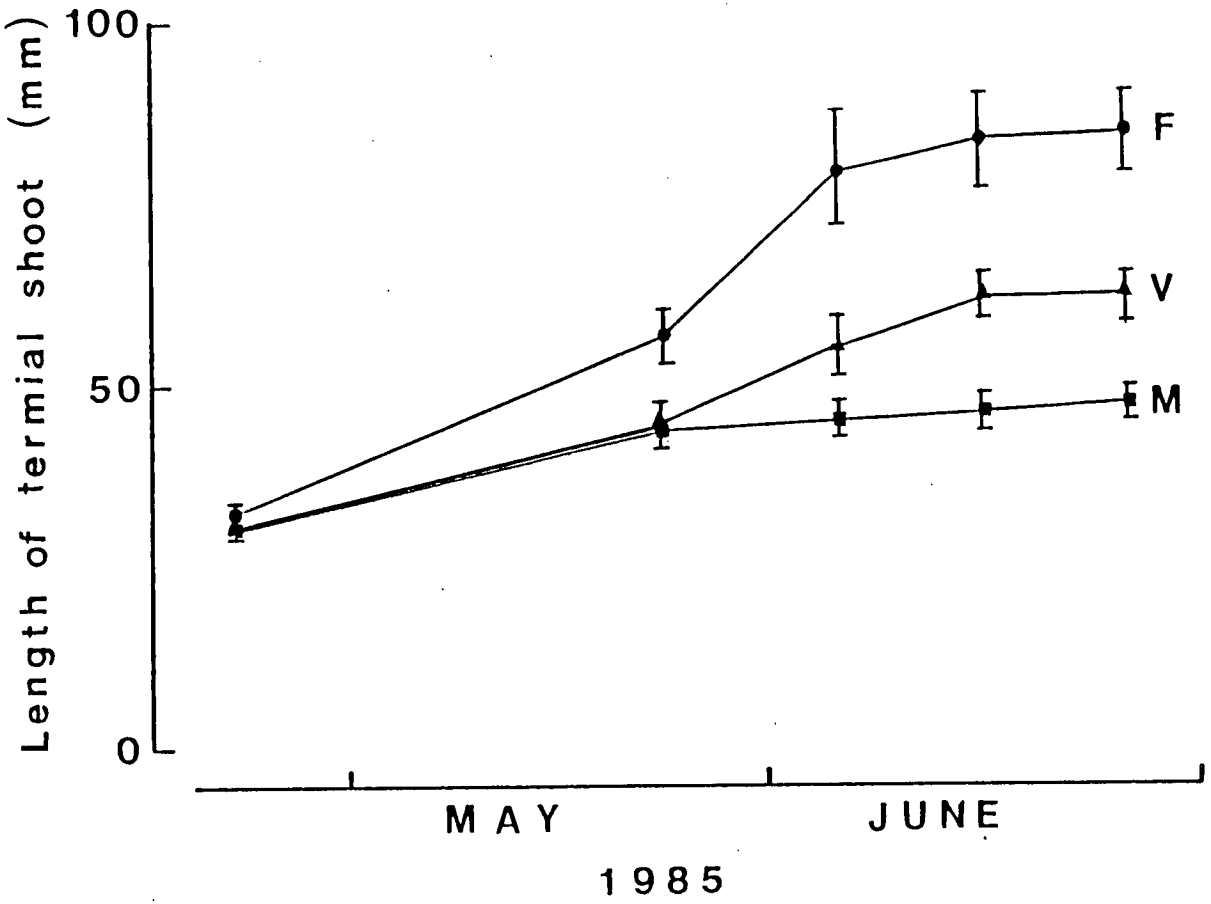


Figure 3.4 Elongation of the terminal shoot of the current year's growth on vegetative (V), male (M) and female (F) cone-bearing branches of P. contorta (mean of 10 shoots ± one standard error).

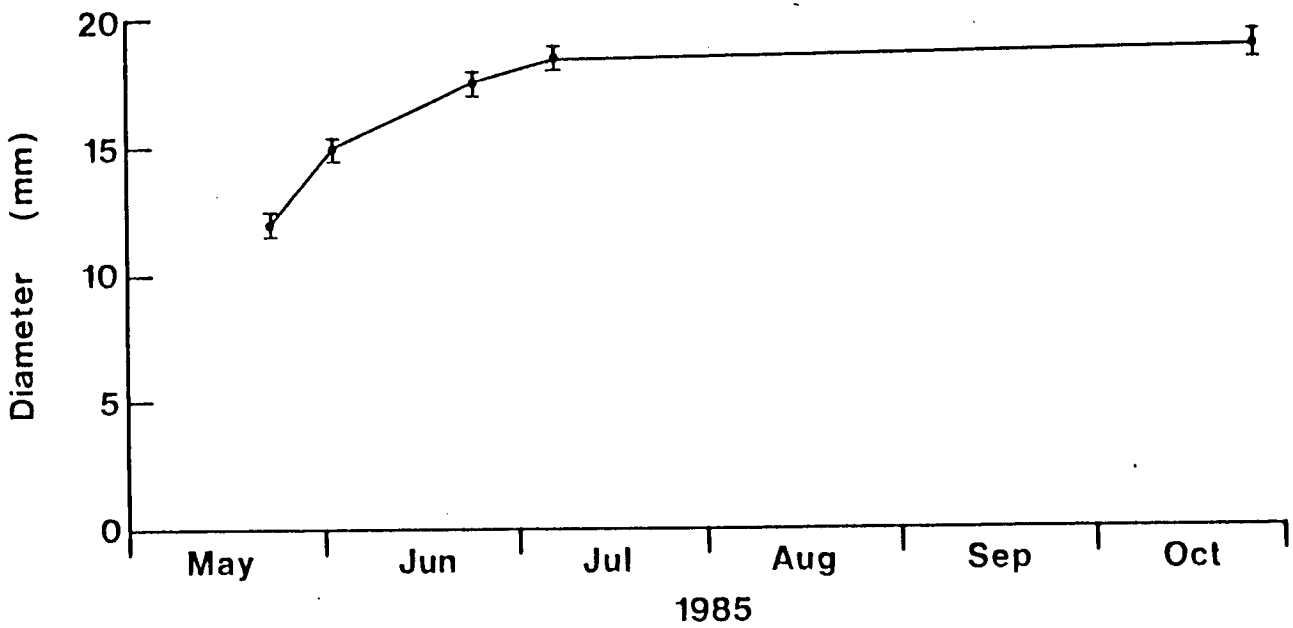


Figure 3.5 Diameter of n2 female cones from May to October 1985 on P. contorta trees

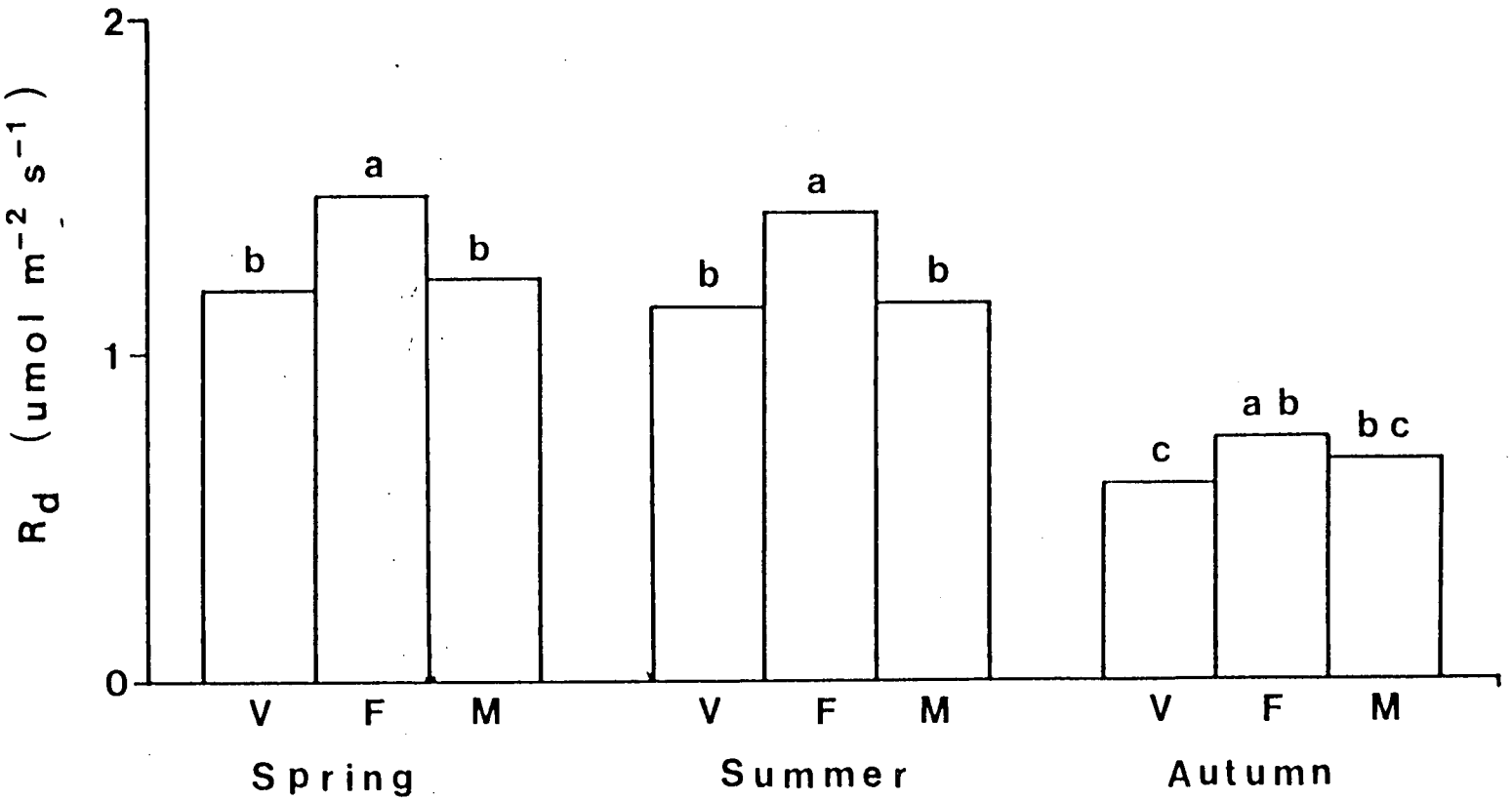


Figure 3.6 Mean dark respiration rates of needles on the parent shoot of vegetative (V), male (M) and female (F) cone-bearing branches of P. contorta, at three sample times. Columns headed with a different letter are significantly different (p = 0.005).

3.5.3 PHOTOSYNTHESIS

The values predicted by the theoretical model correspond reasonably with the observed values (Figures 7,8 & 9). These figures also show that each branch type was sampled over an equivalent range of quantum flux densities at each sample time. Both the spring and summer sample periods had quantum flux densities up to 1800 μmol m^{-2} s^{-1} while in the autumn sample period the highest quantum flux density measured was only around 1000 μmol m^{-2} s^{-1}.

There was more variation between sample periods than between branch types in all of the estimated parameters (Table 3.4).

Table 3.4 Estimated parameter values from the theoretical model (Jarvis et. al. 1985, see text) and the maximum assimilation rates (A_{max} μmol m^{-2} s^{-1}) for the needles on the parent shoots of vegetative, male and female cone-bearing branches of P. contorta at three sample periods (α = initial slope of A/Q curve, g_m = mesophyll conductance (mmol m^{-2} s^{-1}), R_d = dark respiration (μmol m^{-2} s^{-1}), Θ = convexity coefficient).

Branch type	α	g_m	R_d	Θ	A_{max}
Spring sample period					
Vegetative	0.02748	26.68	1.00	0.00	4.93
Female	0.02910	29.04	1.29	0.00	5.71
Male	0.03263	28.52	1.00	0.00	4.93
Summer sample period					
Vegetative	0.05704	62.33	1.17	0.00	9.60
Female	0.04973	75.86	1.43	0.00	10.60
Male	0.05888	70.30	1.18	0.00	8.69
Autumn sample period					
Vegetative	0.05989	64.73	0.60	0.00	9.98
Female	0.07314	61.31	0.76	0.00	9.37
Male	0.07088	61.29	0.69	0.39	10.05

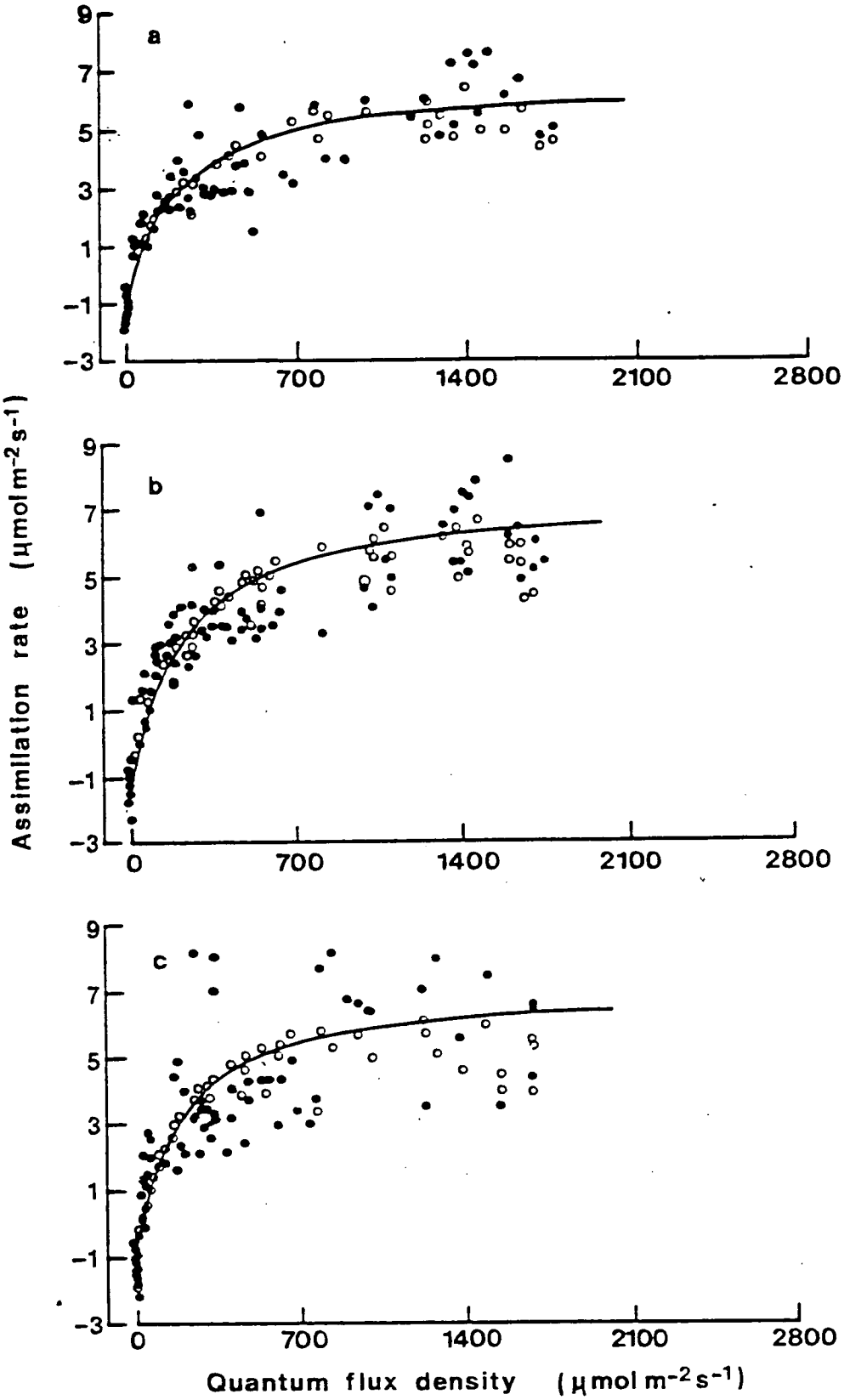


Figure 3.7 Fitted (O) and observed (●) values for the assimilation rates of needles on parent shoots of a) vegetative, b) female and c) male cone-bearing branches on P. contorta at the spring sample period. Lines drawn using parameters estimated from the model of Jarvis et al. (1985) with the mean stomatal conductance.

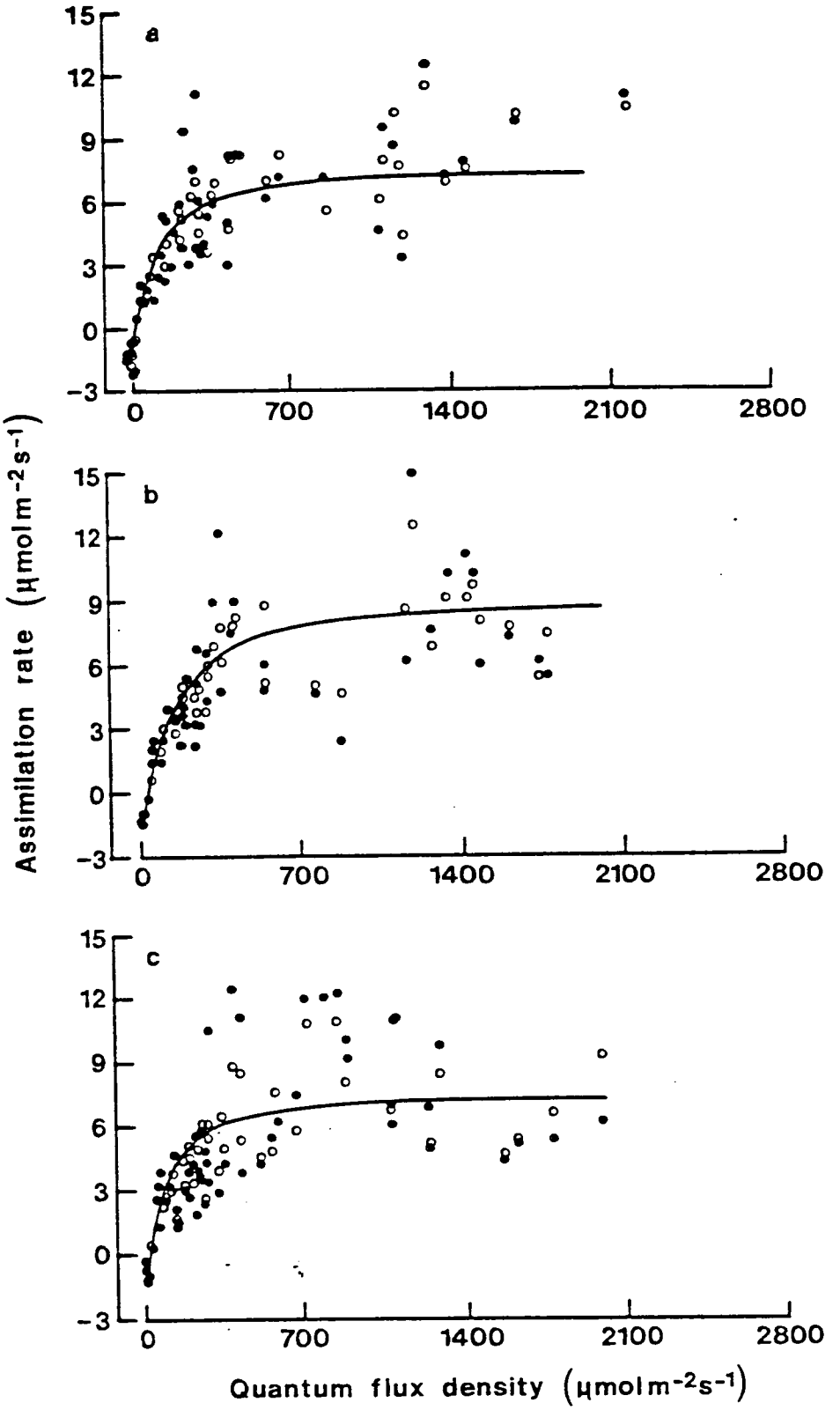


Figure 3.8 Fitted (O) and observed (●) values for the assimilation rates of needles on parent shoots of a) vegetative, b) female and c) male cone-bearing branches on P. contorta at the summer sample period. Lines drawn using parameters estimated from the model of Jarvis et al. (1985) with the mean stomatal conductance.

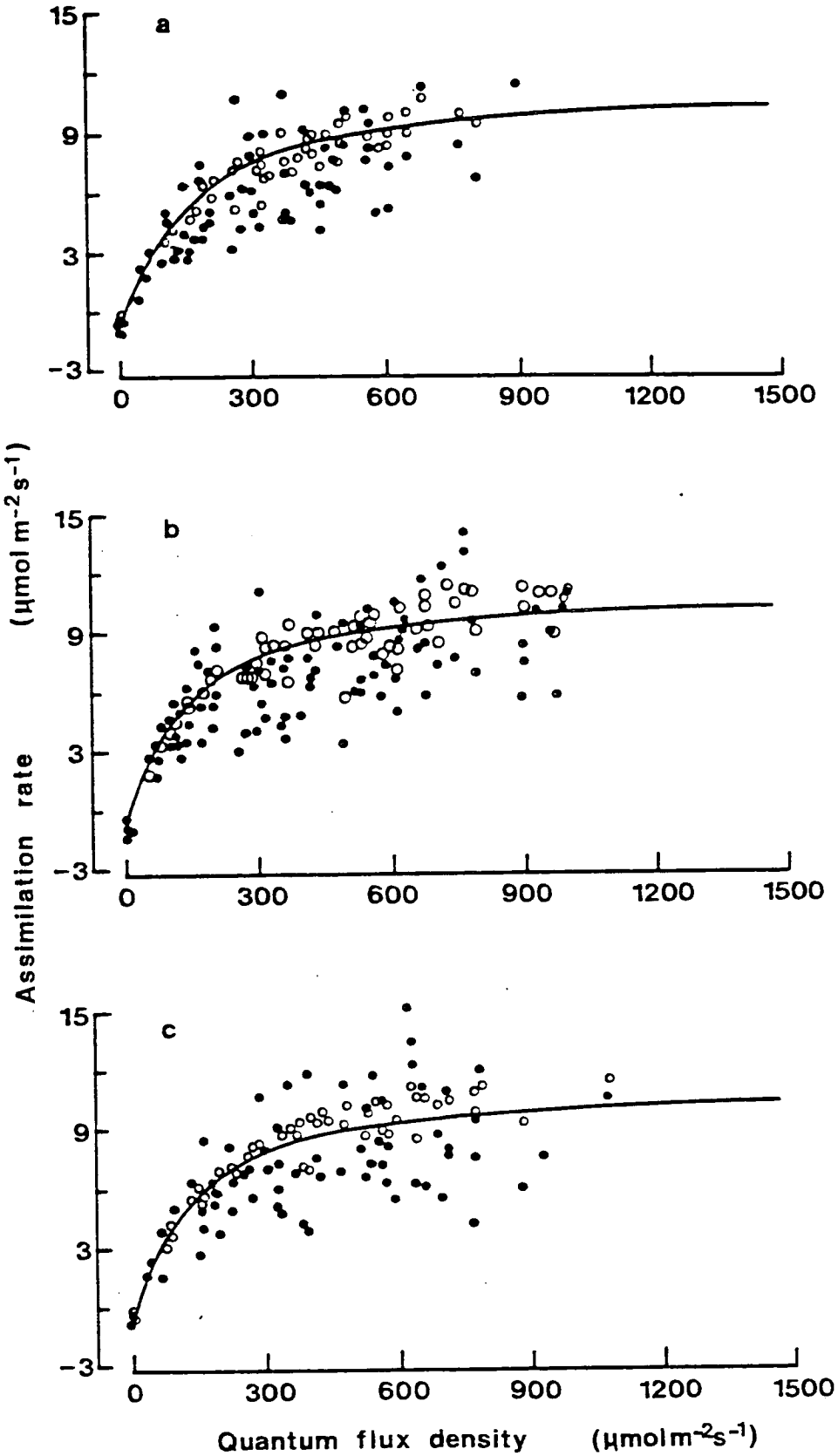


Figure 3.9 Fitted (O) and observed (●) values for the assimilation rates of needles on parent shoots of a) vegetative, b) female and c) male cone-bearing branches on P. contorta at the autum sample period. Lines drawn using parameters estimated from the model of Jarvis et al. (1985) with the mean stomatal conductance.

For convergence of the model the value of θ fell to zero for all branch types in the spring and summer sample periods. As this is lower than published values the model was run with θ fixed at a range of values from 0 to 1, in steps of 0.1, to explore the influence of the θ parameter. All branch types, at the spring and summer sample period, showed the same trend of increasing mean square error with increasing θ (see example of spring sample period Figure 3.10). In the autumn sample period the vegetative and female cone-bearing branches had a θ of zero but the male cone-bearing branches had an estimated θ of 0.39.

The response curves fitted to each branch type (Figure 3.11) were significantly different in the three sample periods (Table 3.5). All three branch types responded to the changing seasons in the same pattern. The lowest A_{max} was measured in the spring sample period, and the highest α was measured in the autumn sample period.

The plots of the predicted values of A against Q (using the appropriate parameter values and a g_s of 100 mmol m^{-2} s^{-1}) show three distinct curves representing the vegetative, male and female cone-bearing branches in each sample time (Figure 3.11). The analysis of the curves for each sample period shows that there are significant differences between the three branch types in the summer and autumn sample periods (Table 3.6). However, in the summer sample period none of the pair-wise comparisons between the curves were significantly different. In the autumn sample period the predicted A/Q curve of male cone-bearing branches was significantly different from that for the vegetative and female cone-bearing branches (Table 3.6). The male cone-bearing branches had a higher assimilation rate for a given quantum flux density.

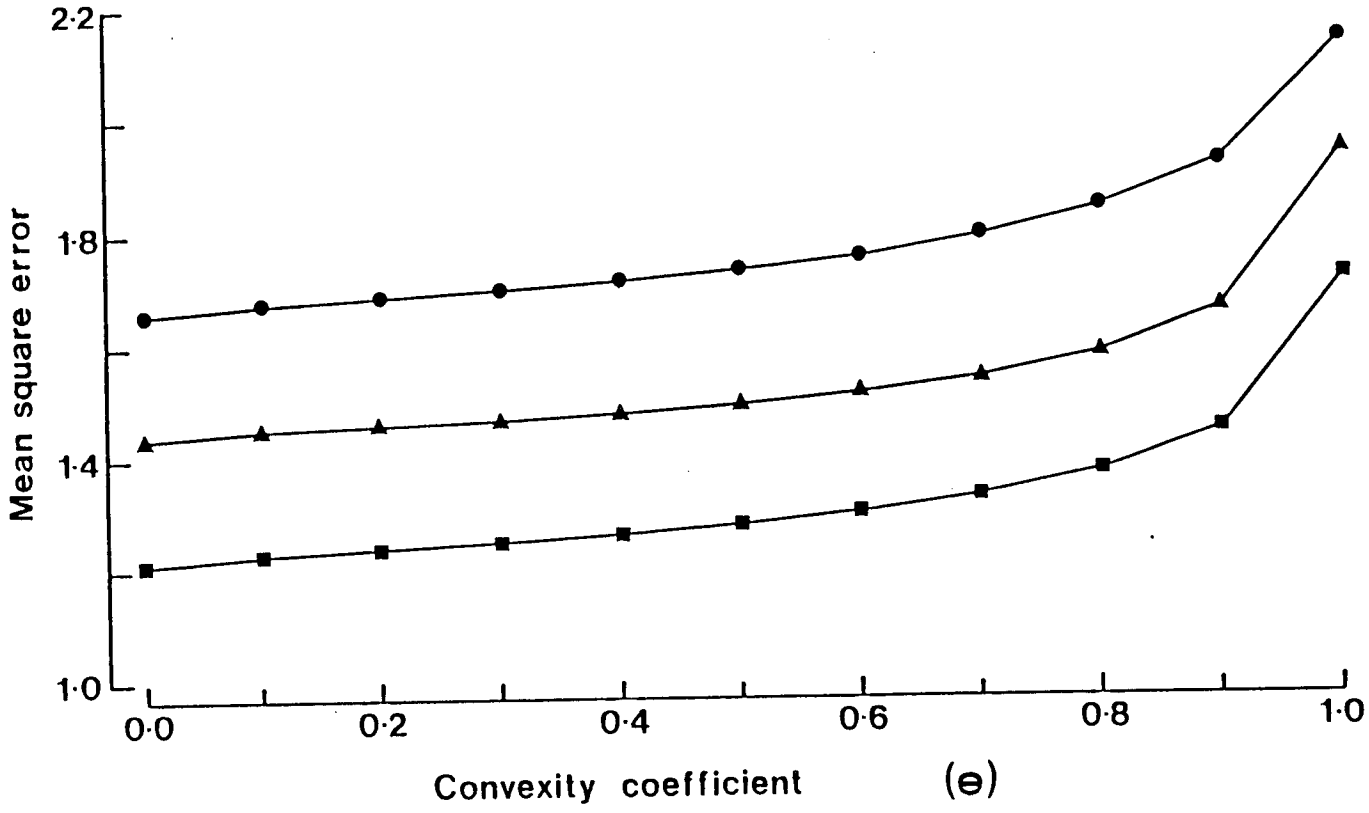


Figure 3.10 Mean square error of vegetative (■) male (●) and female (▲) cone-bearing branches of P. contorta after fitting the theoretical model of Jarvis et al. (1985) with convexity fixed at values from 0 to 1.

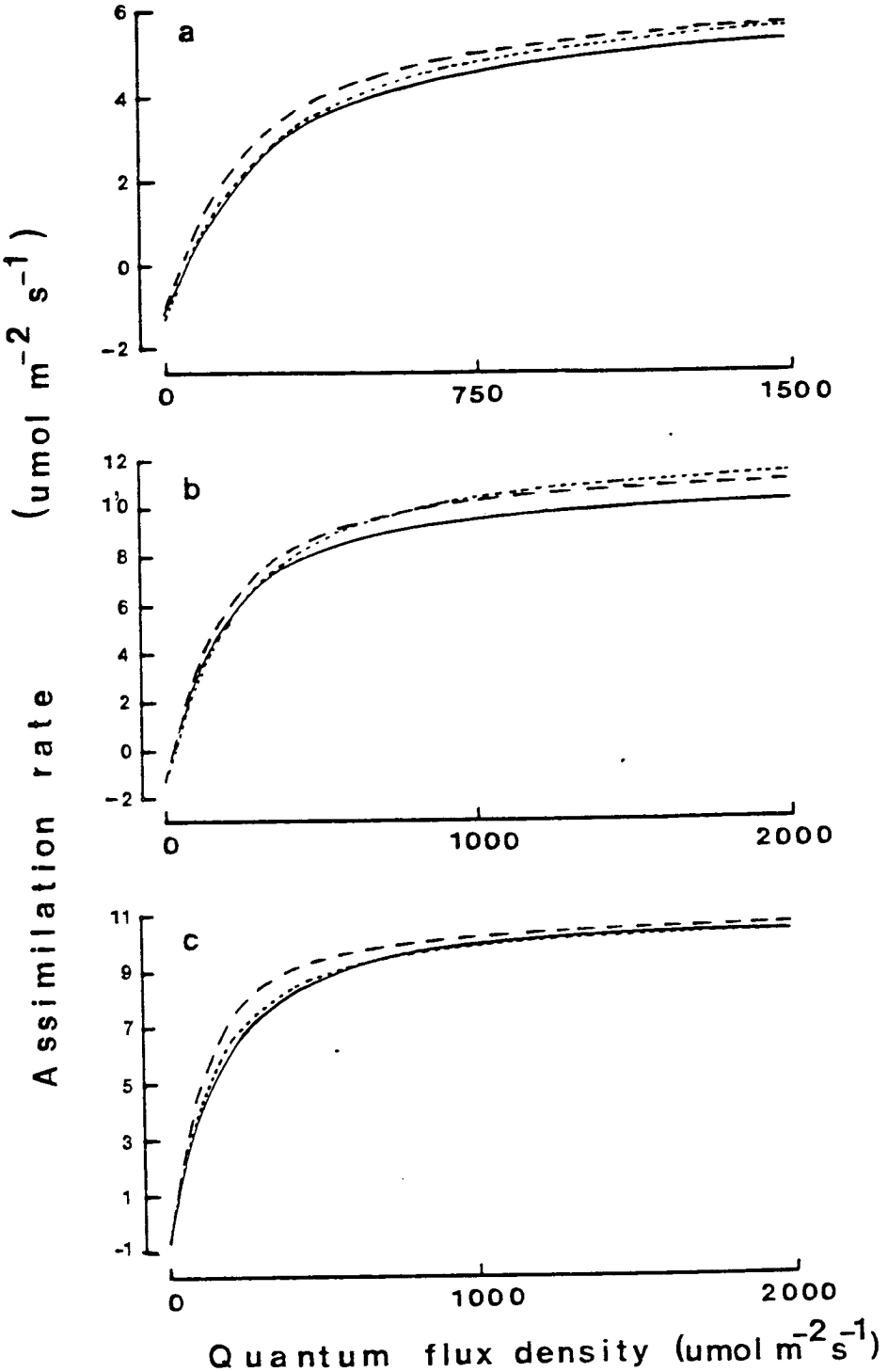


Figure 3.11 Relationship between assimilation rate and quantum flux density for needles on the parent shoot of vegetative (_____), male (----) and female (....) cone-bearing branches of P. contorta sampled in a) spring, b) summer and c) autumn. Lines were drawn using parameters estimated from the model of Jarvis et al. (1985) with a stomatal conductance value of 100 mmol m^{-2} s^{-1}.

Table 3.5 Combined curve analyses of variance tables (Ross 1981), for curves fitted using the theoretical model of Jarvis et. al.: (1985), to the CO_2 exchange rates of needles on the parent shoot of (i) vegetative, (ii) male and (iii) female cone-bearing branches of P. contorta in three sample periods.

Curve comparisons	Degrees of freedom	Sum of squares	Mean sum of squares	F ratio	P value
(i) vegetative branches					
spring vs summer	3	246.40	82.10	81.31	<0.001
residual	198	163.46	0.82		
spring/autumn	3	476.60	158.87	157.76	<0.001
residual	264	292.70	1.11		
summer/autumn	3	37.94	12.65	11.63	<0.001
residual	284	290.96	1.02		
(ii) male cone-bearing branches					
spring vs summer	3	241.46	80.49	79.07	0.001
residual	204	290.05	1.42		
spring vs autumn	3	659.53	219.85	218.10	0.001
residual	270	351.63	1.30		
summer vs autumn	3	97.71	32.57	31.34	0.001
residual	274	337.21	1.23		
(iii) female cone-bearing branches					
spring vs summer	3	300.13	100.04	99.01	0.001
residual	195	200.33	1.03		
spring vs autumn	3	644.53	214.84	213.54	0.001
residual	270	351.63	1.30		
summer vs autumn	3	86.44	28.81	27.54	0.001
residual	279	353.36	1.27		

Table 3.6 Combined curve analyses of variance tables (Ross 1981), for curves fitted using the theoretical model of Jarvis et. al. (1985), to the CO_2 exchange rates of needles on the parent shoot of vegetative (veg), male and female cone-bearing branches of P. contorta at three sample periods.

Curve comparisons	Degrees of freedom	Sum of squares	Mean sum of squares	F ratio	P value
(i) differences between vegetative, male and female cone-bearing branches in each sample period					
spring	6	7.18	1.20	1.30	ns
residual	274	227.76	0.92		
summer	6	11.0	1.83	2.41	<0.05
residual	386	292.77	0.76		
autumn	6	21.17	3.53	3.53	<0.001
residual	469	468.50	1.00		
(ii) differences between the three branch type in the summer					
vegetative vs male	3	3.94	1.31	0.44	ns
residual	221	191.73	0.87		
vegetative vs female	3	6.28	2.10	1.23	ns
residual	211	181.89	0.86		
male vs female	3	6.14	2.05	1.06	ns
residual	214	211.91	0.99		
(iii) differences between the three branch types in the autumn					
vegetative vs male	3	18.83	6.18	6.00	<0.001
residual	305	313.70	1.03		
vegetative vs female	3	3.17	1.06	1.16	ns
residual	323	294.57	0.91		
male vs female	3	10.30	3.43	3.24	<0.05
residual	310	328.68	1.06		

3.6 DISCUSSION

All three branch types exhibited similar seasonal variation in net assimilation rates. Seasonal trends in assimilation rates have been observed by many workers and are considered partly the result of seasonal variation in the weather (particularly temperature and quantum flux density) and partly the result of biological processes which are dependant upon the season (e.g. the winter dehardening process, see Jarvis et al. 1976, Linder and & Troeng 1980). The low assimilation rates in the early spring i.e. before pollen was released, may have been the result of low winter temperatures. Troeng & Linder (1982a) have shown that one-year-old needles of Scots pine may take up to three months to recover full photosynthetic capacity in the spring. During the summer and autumn environmental variables are usually the most important in controlling the assimilation rate of needles. As a result of the very high rain fall during the summer there was little difference in the values of vapour pressure difference and stomatal conductance measured in this study. The low quantum flux densities in the autumn, may account for the steep initial slope (α) of the A/Q curve which is typical of shade-adapted shoots. There was more variation in the assimilation rates between the sample periods (i.e. seasonal variation) than between the three branch types within a sample period.

There were no significant differences in the models fitted to the vegetative, male and female cone-bearing branches in the spring and summer sample periods. As these two periods cover the time of maximum dry weight increment of the cones, it would appear that male and female cone development does not induce an increased CO_2 exchange rate on the needles nearest to them, as suggested in the introduction (section 3.2). Neales & Incoll (1968) reviewed the hypothesis that the CO_2 exchange rate of leaves was controlled by sink activity and concluded that although there was undoubtedly a relationship of some sort between photosynthesis, leaf carbohydrate content and sink demand there was no conclusive evidence that these factors were causally related.

In the autumn sample period, significant differences in the A/Q curves were found between the needles on the parent shoot of male cone-bearing branches compared to both vegetative and female cone-bearing branches. The male cone-bearing branches had the highest A_{max} and θ. Male cones also resulted in a 30% - 50% reduction in the number of current year's needles (Chapter 2). In the autumn, these needles are known to export assimilates to other parts of the tree (see Ericsson 1980). It is possible, therefore, that the reduction in the number of needles on the current year's growth of male cone-bearing branches results in less assimilate production in the autumn. This may then induce a higher CO_2 exchange rate on older needles of the male cone-bearing branches compared to equivalent vegetative branches. Furthermore, in the autumn the current male bud is growing and creating a strong sink. The influence of plant growth regulators originating in the sink organs on the control of CO_2 exchange rate of associated leaves, was reviewed by Bidwell (1983). He concluded that there was good evidence that such CO_2 regulating mechanisms operate through the production of hormones or hormone-like substances by the photosynthate sinks themselves. Plant growth regulators, particularly auxins and gibberellins, have long been associated with reproductive structures (see reviews by Lee 1979, Ross & Pharis 1985, Owens & Blake 1985). Although the male bud is relatively small in the autumn, it is possible that it may well be causing enhanced rates of assimilation of the needles on the parent shoot. The positive relationship between the total dry weight of the needles on the parent shoot and the total dry weight of current year's growth of male cone-bearing branches in the spring, found in Chapter 2, further support this hypothesis. It is possible, therefore, that the reduction in the current year's needle complement, and the influence of the current male bud, both contributed to the increased assimilation rates of the needles on the parent shoot of male cone-bearing branches.

The assimilation rates of needles on the parent shoot of female cone-bearing branches were not significantly higher than closely associated vegetative branches at any time during the measurement period. However, as the vegetative branches were always physically

very close to the female cone-bearing branches in this study it is possible that n2 female cones produced a plant growth regulator (see Bidwell 1983) which induced a higher CO_2 exchange rate on the vegetative branches immediately adjacent (see Discussion in Chapter 2). In order to study the influence of cones independently from the rest of the tree a simple potted plant system was devised (Chapter 6).

The convexity coefficient (θ) was estimated as zero for all branch types, in all sample periods with only one exception (male cone-bearing branches in the autumn). A zero value for θ indicates that a rectangular hyperbola is an adequate fit to the data. Values of θ between 0.4 and 0.9 have been found, when needles have been removed from the shoot to avoid self-shading of the remaining needles (Leverenz & Jarvis 1979 Leverenz 1987, 1988). However lower values of θ have been found for intact shoots. This has been attributed to the steep gradient of light between photosynthesising cells within a needle which results from the long path lengths of the light beams passing obliquely through needles, especially through needles overlapping each other in the intact shoot (see Leverenz & Hinckley 1989). The male cone-bearing branches in the autumn sample period had an estimated θ of 0.39, which suggests that the needles on male cone-bearing branches were less affected by self-shading in the autumn than in the spring and summer. It is possible this resulted from the different orientation of the sun relative to the shoots in the autumn but further work is be needed to confirm this observation.

In the spring and summer, needles on the parent shoot of female cone-bearing branches had higher dark respiration rates than needles on the vegetative and male cone-bearing branches. Dark respiration rates have been shown to be high during the elongation of the current year's shoot (Clark 1961, Negisi 1966, Drew & Ledig 1981), and this has been attributed to an increase in the so-called 'growth' component of respiration (see Penning de Vires 1975). Thus the increase in dark respiration rates of needles on the parent shoot of female cone-bearing branches, may perhaps be attributed to their significantly longer terminal shoot compared with both the

vegetative and male cone-bearing branches. The presence of nearby n2 female cones may also contribute to the increase in dark respiration rates as both the female cones and the current year's shoots grow during the same period.

In conclusion, it would appear from this study that branches bearing n2 female cones have increased carbon loss compared with equivalent vegetative branches, and this may be attributable to more shoot elongation as well as the growth of n2 female cones. In contrast, the needles on the parent shoot of male cone-bearing branches have increased carbon gain in the autumn, and this may be the result of: (i) male cones in the developing bud creating a strong sink for assimilates and (ii) the current year's male cones reducing the photosynthetic area of the current year's shoot. Together these two possibilities may account for the increased assimilation rates in the needles of the parent shoot of male cone-bearing branches. The current year's needles may similarly have an increased CO_2 exchange rate: this hypothesis was tested in the following chapter under controlled environmental conditions.

3.7 SUMMARY

1) In the autumn, needles on the parent shoot of male cone-bearing branches had significantly higher photosynthetic rates compared to needles on vegetative and female cone-bearing branches. There were no significant differences between the assimilation rates of needles on vegetative and female cone-bearing branches in any of the three sample periods (spring, summer or autumn).

2) It was suggested that needles on male cone-bearing branches had higher autumn assimilation rates because of: (i) reduced needle complement on the current years growth and (ii) the presence of developing male cone buds.

3) Needles on all three branch types had significantly different photosynthetic rates at the three sample times.

4) The seasonal differences in the CO_2 exchange rates of the needles on the parent shoots were thought to be caused by the normal seasonal trends associated with conifers in temperate climates.

5) Needles on female cone-bearing branches had higher respiration rates than needles on vegetative branches, at all three sample times. Needles on female cone-bearing branches also had significantly higher respiration rates than needles on male cone-bearing branches in the spring and summer sample periods but there were no significant difference at the autumn sample period.

6) It is suggested that the higher dark respiration rates associated with the female cone-bearing branches were the result of the simultaneous growth and extension of both the n2 female cone and the current year's shoot. Female cone-bearing branches also had significantly longer terminal shoots in the current year compared with the vegetative and male cone-bearing branches.

CHAPTER 4

INFLUENCE OF MALE CONES ON THE RATES OF NEEDLE PHOTOSYNTHESIS AND RESPIRATION IN CONTROLLED CONDITIONS

4.1 AIM

It was the aim of this study to test the hypothesis that in the autumn male cones on the terminal shoot of the current year's growth induce a higher CO_2 exchange rate in the needles of the parent shoot and the shoot which bore them.

4.2 INTRODUCTION

In field measurements, the autumn assimilation rates of needles on the parent shoot of male cone-bearing branches were found to be significantly higher for a given quantum flux density, compared with needles on the parent shoot of vegetative branches (Chapter 3). It was suggested that this increased CO_2 exchange rate resulted from increased sink activity of the developing male bud and reduction in needle number on the terminal shoot of the current year's growth of male cone-bearing branches (see section 3.6 for full discussion). By the same reasoning it is also possible, that the assimilation rate may be higher in the remaining current year's needles on the terminal shoot of male cones-bearing shoots compared with equivalent vegetative shoots. It was the aim of this study to quantify, under standard environmental conditions, the CO_2 exchange rates of needles on the parent and terminal shoot of the current year's growth and to determine the influence of male cones on the CO_2 exchange rates of the shoots which bear them.

Potted plants were used in this study rather than branches as used in Chapter 3, because they are more discrete. Ideally four sets of plants are required to separate the influence of male cones on the current year's shoot and in the developing bud on the assimilation rate of associated needles. They are plants with:
(i) vegetative shoot and male cone-bearing bud,
(ii) male cone-bearing shoot and male cone-bearing bud,

(iii) vegetative shoot and vegetative bud, and
(iv) male cone-bearing shoot and vegetative bud.

Unfortunately there were no suitable plants of types (iii) and (iv) available for this study. Therefore the CO_2 exchange rate of needles on the parent shoot and the terminal shoot of the current year's growth was measured on plants of type: (i) and (ii) to determine the influence of a reduction in the needle complement of the current year's growth as a result of the presence of male cones on the assimilation rate of associated needles.

4.3 MATERIALS

In September 1987 six plants were selected from a set a P.contorta cuttings, struck in February and potted into 0.23 dm^3 containers in mid May 1985. The cuttings were potted-on (1.5 dm^3 containers) in February 1986 and placed out of doors in the I.T.E. nursery, Bush Estate, near Edinburgh. The parent trees were themselves cuttings from Shin near Lairg, Scotland (Forestry Commission clone number 103/4). They were planted in 1976 at the nursery of the Northern Research Station of the Forestry Commission, Roslin, Edinburgh, Scotland. The provenance of this material was south coast of Washington State, USA. Three of the plants chosen had produced cones in 1987, and these will be termed the male cone-bearing plants (nos. 701-703). Vegetative plants (nos. 601-603) had not produced any male cones in 1987. However, all six plants had male cones developing in the terminal bud of the current year's growth (i.e. male cones which would shed pollen in spring 1988).

Some needles were removed from the plants to allow the shoots to be enclosed in the chamber. The number and area of these needles and the needles enclosed in the leaf chamber were measured on a leaf area meter (Model 3100, LiCor Inc., Lincoln, Nebraska, USA). The lengths of the current year's terminal shoot and the parent shoot were measured with a ruler (Table 4.1).

Table 4.1 Number, area and dry weight of the needles and the length of the (i) parent shoots and (ii) current year's terminal shoots of vegetative (veg) and male cone-bearing (male) plants of P.contorta removed prior to measurment and enclosed in the leaf chamber.

			NEEDLE PAIRS IN THE CHAMBER				NEEDLE PAIRS REMOVED PRIOR TO MEASURMENTS			
Plant type	Plant number	Date of CO2 measurement	Number needle pairs	Total area (cm2)	Total weight (g)	Internode distance (mm)	Number needle pairs	Total area (cm2)	Total weight (g)	Total length of shoot (mm)
(i) Parent shoot										
Veg	601	27/9/87	52	42.3	1.24	0.58	35	25.3	0.85	78
Veg	602	29/9/87	44	37.9	1.07	0.57	35	27.7	0.96	70
Veg	603	1/10/87	37	17.7	0.48	0.54	34	17.4	0.52	55
Male	701	26/9/87	83	79.6	2.30	0.60	41	36.8	1.12	140
Male	702	28/9/87	48	53.4	1.55	0.94	62	57.9	1.88	144
Male	703	30/9/87	81	49.5	1.32	0.68	13	9.1	0.30	102
(ii) Terminal shoot of the current year's growth										
Veg	601	16/9/87	119	99.3	2.57	0.59	28	22.7	0.65	98
Veg	602	18/9/87	82	51.4	1.26	0.65	45	25.5	0.64	78
Veg	603	21/9/87	71	58.2	1.57	0.59	32	25.7	0.76	72
Male	701	17/9/87	111	84.1	2.03	0.43	0	0	0	76
Male	702	19/9/87	126	118.6	2.68	0.55	0	0	0	108
Male	703	20/9/87	83	84.3	2.28	0.51	0	0	0	69

4.4 METHODS

An open gas exchange system, described by Sandford (1984, 1987), was used. The system, housed in a temperature-controlled room, (Figure 4.1) allows the user to control the air flow, leaf temperature, water vapour pressure difference across the leaf surface (D_l), ambient CO_2 concentration (C_a), and the average intercellular CO_2 concentration (C_i) of the needles within the chamber.

The leaf chamber was designed and built for this study (Figure 4.2). It was constructed of aluminium (Aalco (Edinburgh) Ltd, West Harbour Road, Granton, Scotland), and coated with nickel by the process of electroplating (Precision Machine (Edinburgh Ltd), Newbattle Industrial Estate, Dalkeith, Scotland). Two six volt fans (Micronel V241L, Radiatron Components Ltd, Twickenham, Middlesex, England) were mounted through a nickel-plated aluminium sheet which formed a baffle near the base of the chamber. Air entered the chamber by a hole beside the fans and was directed to the top of the chamber. The sample gas was extracted from a hole near the base of the chamber above the baffle (Figure 4.2). Double-glazed windows were inserted in two walls of the chamber. The quantum flux density in the chamber was measured by two photodiodes (BPW21, RS Components Ltd, PO Box 99, Corby, Northants, England).

The leaf chamber was bilaterally illuminated with metal-halide lamps (HQI, 400 W, Wotan Ltd., London, England). The light emitted from each lamp was collimated by an aspheric Fresnel lens made from acrylic plastic with a focal length 31.8 cm (Ealing Electro-optic plc, Watford, England). The quantum flux density in the chamber was controlled with neutral density filters (Frew-Smith Ltd, Irvine, Scotland).

The leaf temperature, air temperature and chamber wall temperature were measured by copper/constantan thermocouples (SWG 38, Dural Plastics & Eng. Pty. Ltd., Dural, Australia). The chamber air flow was controlled by a Tylan mass flowmeter (Model FC261, Tylan Corporation, Surrey, England). The leaf temperature was controlled

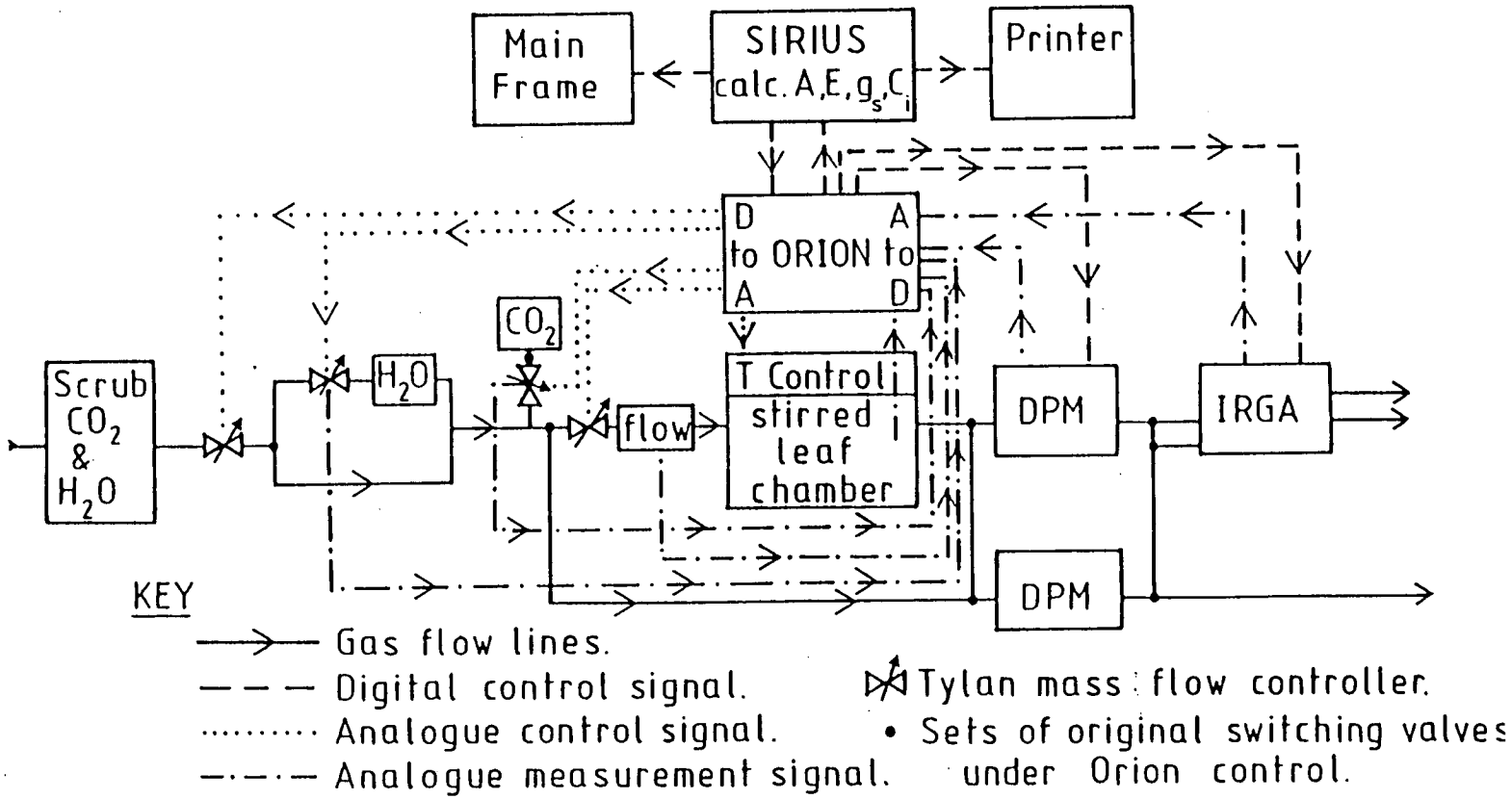


Figure 4.1 Block diagram of open pathway system used to measure CO_2 exchange of needles on the terminal and parent shoot of vegetative and male cone-bearing plants of P. contorta.

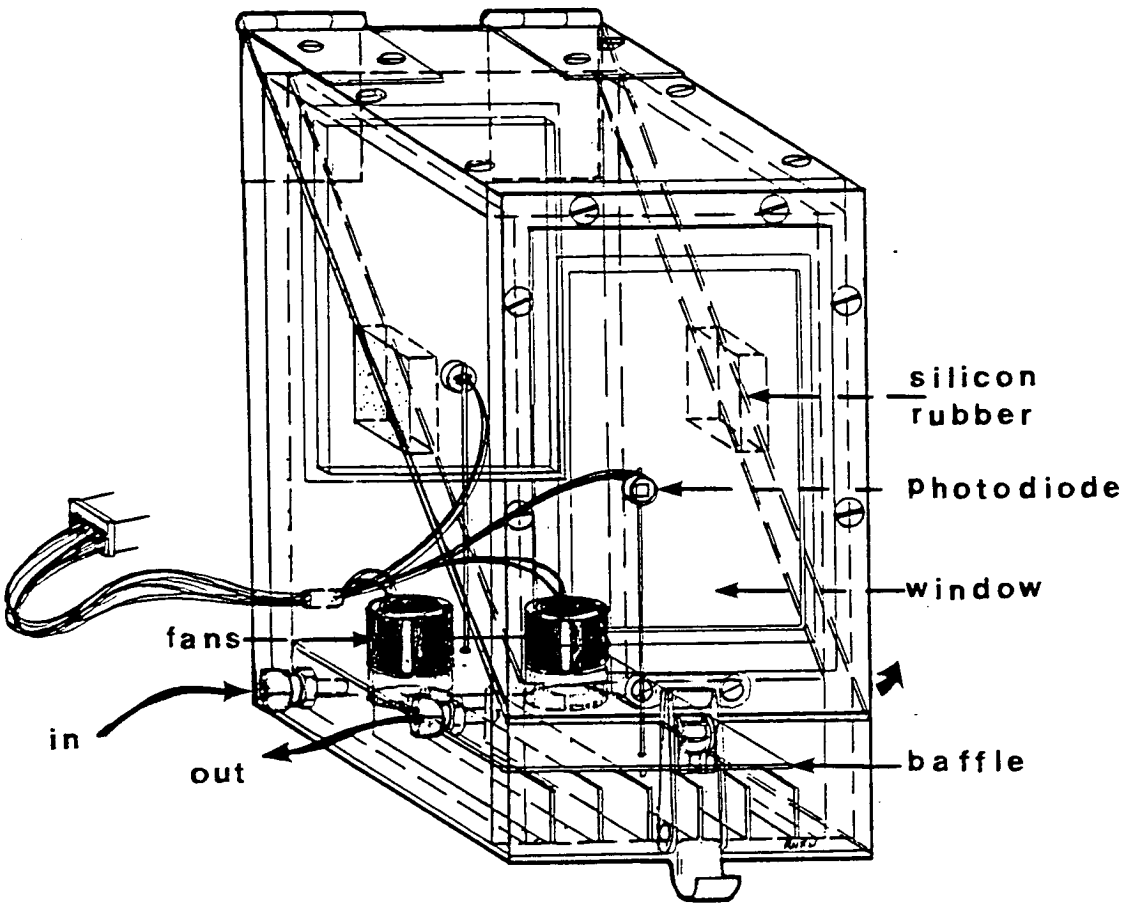


Figure 4.2 Diagram of the leaf chamber used to measure the CO_2 exchange rate of needles on parent and terminal shoots of the current year's growth of vegetative and male cone-bearing plants of P. contorta.

by a modified temperature control system (Model 031, Eurotherm Ltd, Sussex, England). This took the signal from a thermocouple attached to a needle surface and adjusted the leaf temperature by varying the driving voltage to a Peltier cooling unit (Model 803-1010-01, Cambion, Castleton, England) beneath the leaf chamber.

The ambient vapour pressure (D_a) and CO_2 concentration (C_a) in the leaf chamber, were controlled via three Tylan mass flowmeters, a dewpoint hygrometer (Series 3000, Michell Instruments Ltd. Cambridge, England) and an infra-red gas analyser (URAS 3E, Hartmann & Braun (U.K) Ltd, Northampton, England). The air was extracted from the laboratory by a compressor (Model NE 037.2 AN 10, Knf Ltd, Wantage, England). Water and CO_2 were removed by passing the air through three columns of soda lime, three columns of silica gel and one further column of soda lime and Drierite. One mass flowmeter initially controlled the flow of the dry CO_2-free air. Some of the air, controlled by the second mass flowmeter, was diverted through a humidifier, controlled at a constant temperature of 32.0 ^{o}C, and was afterwards remixed with the remainder of the dry, CO_2-free air. A third mass flowmeter bled pure CO_2 into the humidified air. Thus the air was humidified and mixed with CO_2, before passing through the reference line to the leaf chamber. D_a was measured by the dewpoint meter and the value was automatically fed into a microcomputer (Sirius 1, Computing Service (Scotland) Ltd, Edinburgh, Scotland), which then calculated D_1 and adjusted the flow rate of the saturated air through the second mass flowmeter until the difference was less than 0.1 kPa between the estimated D_1 and that required. The CO_2 concentration in the chamber (measured with the infra-red gas analyser) was also fed into the microcomputer, which then adjusted the bleeding rate of the pure CO_2 through the third mass flowmeter, so that C_a was controlled at a value close to that required.

All inputs from the sensors and instruments were sent to the microcomputer through an interface (Orion 3530B, Solartron Instrumentation Group, Farnborough, England). The control outputs from the microcomputer also passed through this interface.

The following conditions were controlled within the chamber: leaf temperature 15 °C, C_a 350 μmol mol^{-1}, D_l 0.8 kPa. Each plant was left in the chamber overnight. In the morning the CO_2 exchange rate was recorded before the lights were switched on. The quantum flux density was increased after the assimilation rate was steady for 20 minutes (Figure 4.3).

The theoretical model of Jarvis *et al.* (1985), was fitted to the data for each shoot type as was done in Chapter 3 to compare:
(i) the CO_2 exchange rate of male and vegetative plants measured in this study and
(ii) the parameters estimated from the field study with the parameters estimated in this study (see section 3.4 for description of the model and parameter estimates).

The combined model technique of Ross (1981) was utilised to determine statistical differences in the estimated model parameters. The predicted assimilation rate against quantum flux density curves (A/Q) were drawn using the estimated parameters and a standard g_s value of 100 mmol m^{-2} s^{-1} for all the shoots.

The maximum assimilation rate (A_{max}) was calculated as

$$A_{max} = (C_i - \Gamma)\, g_m$$

where Γ is the CO_2 compensation concentration and was assumed to be 50 μmol mol^{-1}, C_i was the mean C_i for Q values greater than 1000 μmol m^{-2} s^{-1}, and g_m was estimated by fitting the model of Jarvis *et al.* 1985.

4.5 RESULTS

4.5.1 RESPIRATION

The needles on the terminal shoot of the current year's growth of vegetative plants had significantly higher dark respiration rates (R_d) compared with needles on the male cone-bearing plants (Figure 4.4). However, there was no significant difference between the

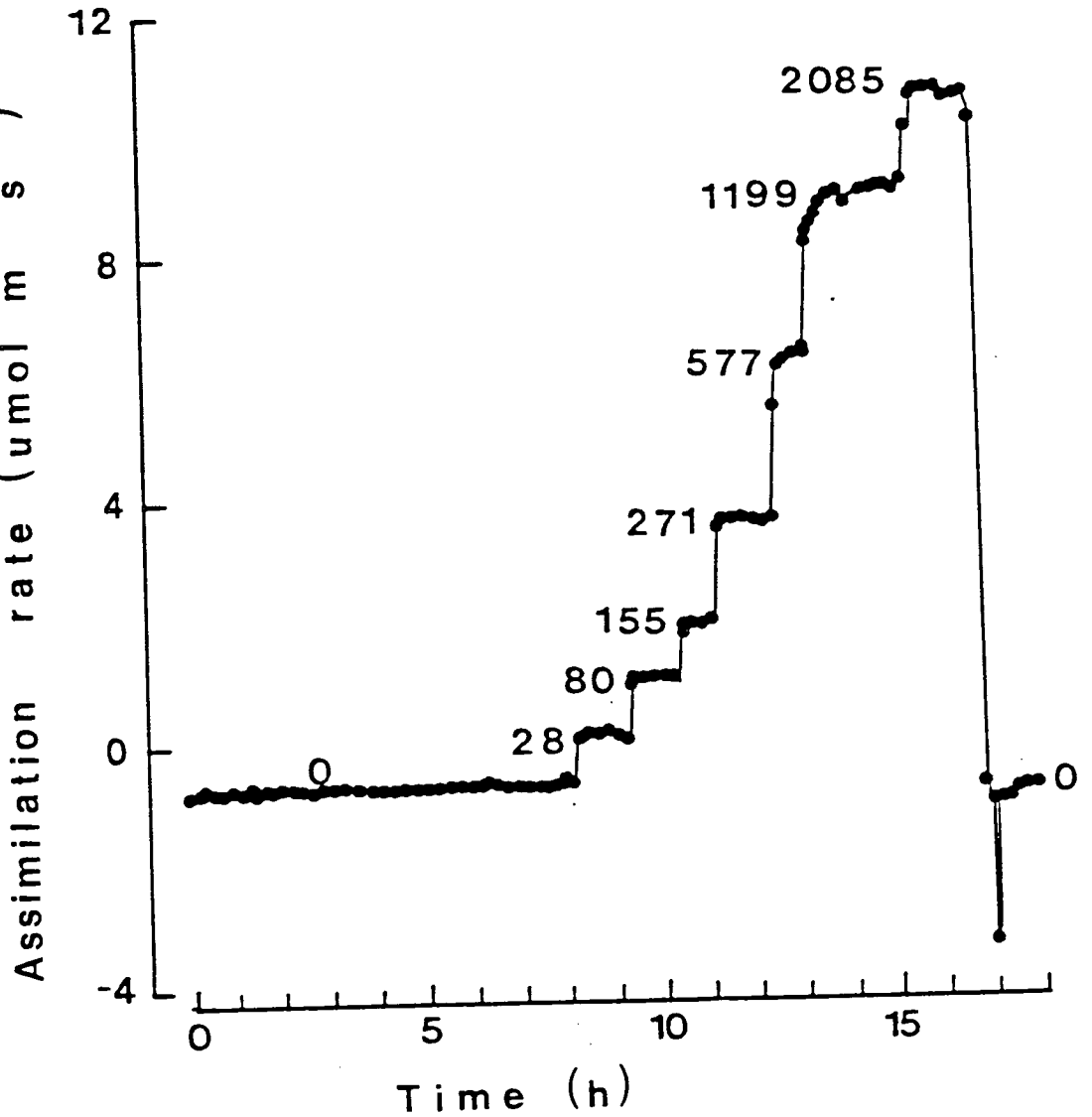


Figure 4.3 Time response of the assimilation rate of plant 702 of P. contorta to quantum flux densities from 0 to 2085 μmol m^{-2} s^{-1}.

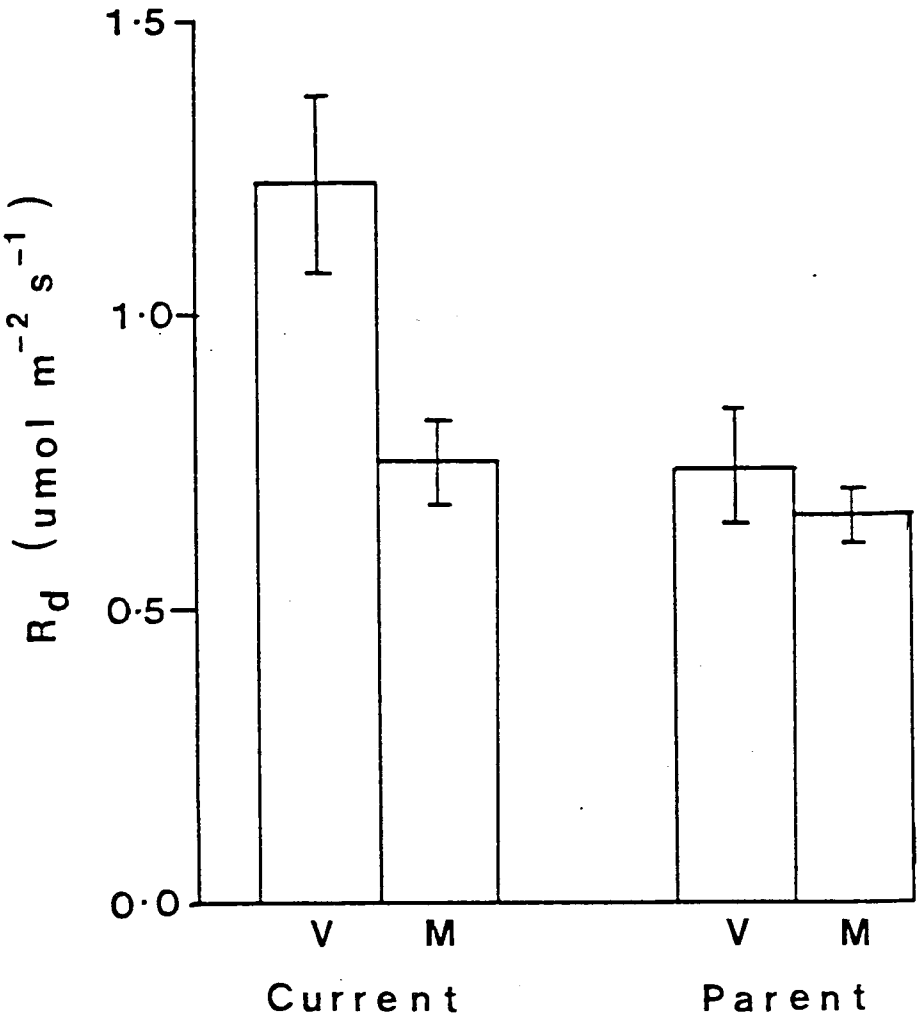


Figure 4.4 Dark respiration rates of the needles on parent and terminal shoots on the current year's growth of vegetative and male cone-bearing plants of P. contorta (± standard error of the mean).

values of R_d for needles on the parent shoots of vegetative and male cone-bearing plants.

Needles on the terminal shoot of the current year's growth of vegetative plants had a higher dark respiration rate compared to the needles on the parent shoot of vegetative plants. There was no significant difference between the dark respiration rates of the needles on the current year's growth and those on the parent shoot of male cone-bearing plants.

4.5.2 PHOTOSYNTHESIS

Needles on the parent shoot of male cone-bearing plants clearly had higher CO_2 exchange rate for a given quantum flux density compared with needles on the parent shoot of vegetative plants (Figure 4.5a). There was not, however, an equivalent difference in stomatal conductance (Figure 4.5b), although male cone-bearing plants generally had a higher stomatal conductances for a given quantum flux density. The assimilation rate / quantum flux density response curve fitted by the model (Figure 4.6) shows that needles on the parent shoot of male cone-bearing plants had a significantly higher (Table 4.2) photosynthetic rate for a given quantum flux density compared to vegetative plants. Male cone-bearing plants had higher A_{max}, α and g_m compared to vegetative plants. (Table 4.3).

There was not such a clear difference between the assimilation rates of needles on the terminal shoot of the current year's growth of male cone-bearing plants and vegetative plants (Figure 4.7a). Needles on the terminal shoot of the current year's growth of male cone-bearing plants did, however, appear to have a consistently lower stomatal conductance compared with the needles on the vegetative plants (Figure 4.7b). Unfortunately, the needles on the terminal shoot of the current year's growth of male cone-bearing plant no. 703 appear to have been stressed in some way prior to being placed in the chamber. The maximum stomatal conductance of plant 703 was much lower compared with the other shoots measured (18 mmol m^{-2} s^{-1} compared with 77 - 209 mmol m^{-2} s^{-1}), and the A_{max} was also much lower (2.3 μmol m^{-2} s^{-1} compared with 5.7 - 11.5). In

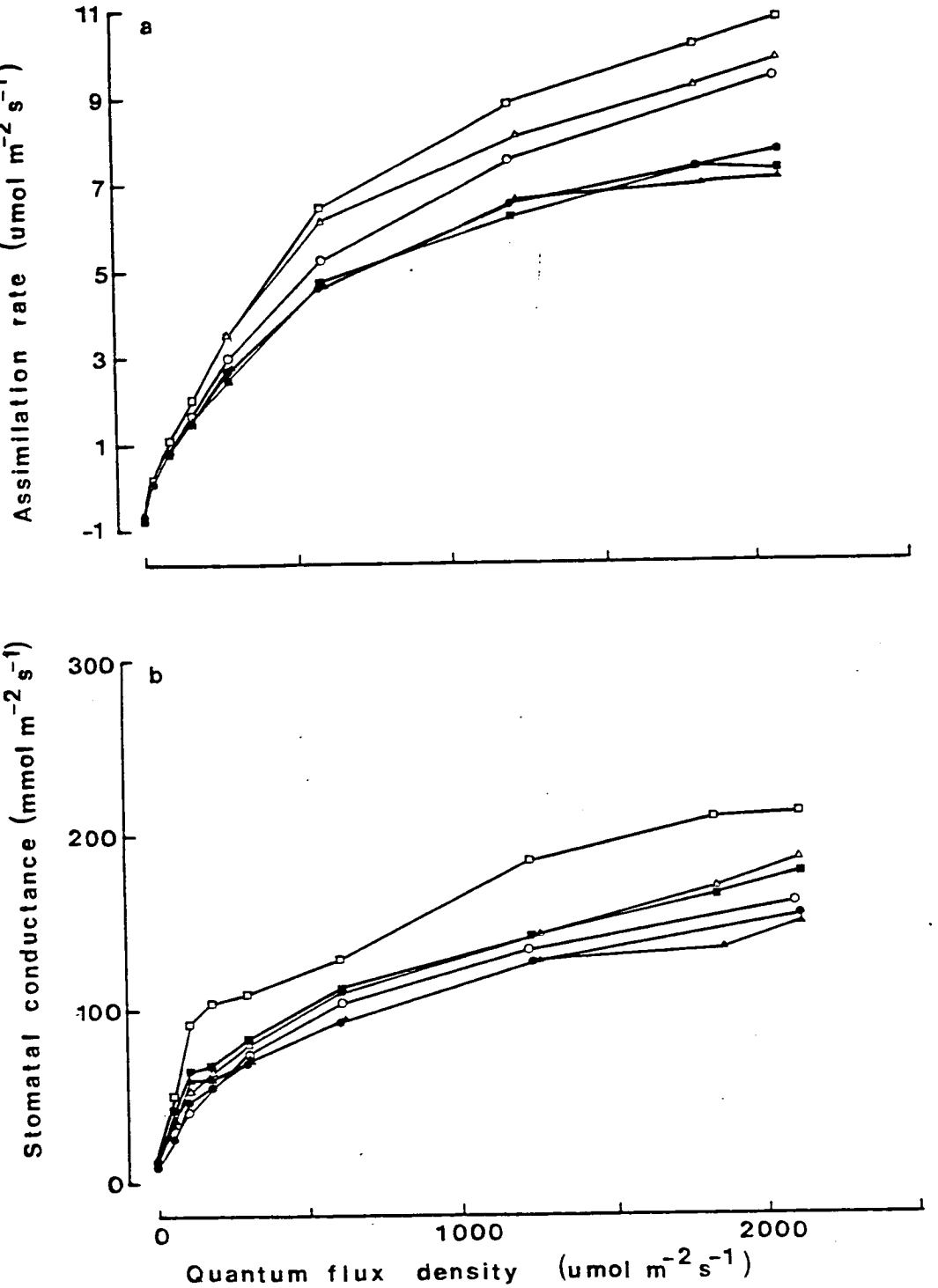


Figure 4.5 The relationship between a) assimilation rate, b) stomatal conductance and quantum flux density for needles on the parent shoot of vegetative (601 ● ,602 ■ ,603 ▲) and male cone-bearing (701 ○ , 702 □ ,703 △) plants of P. contorta.

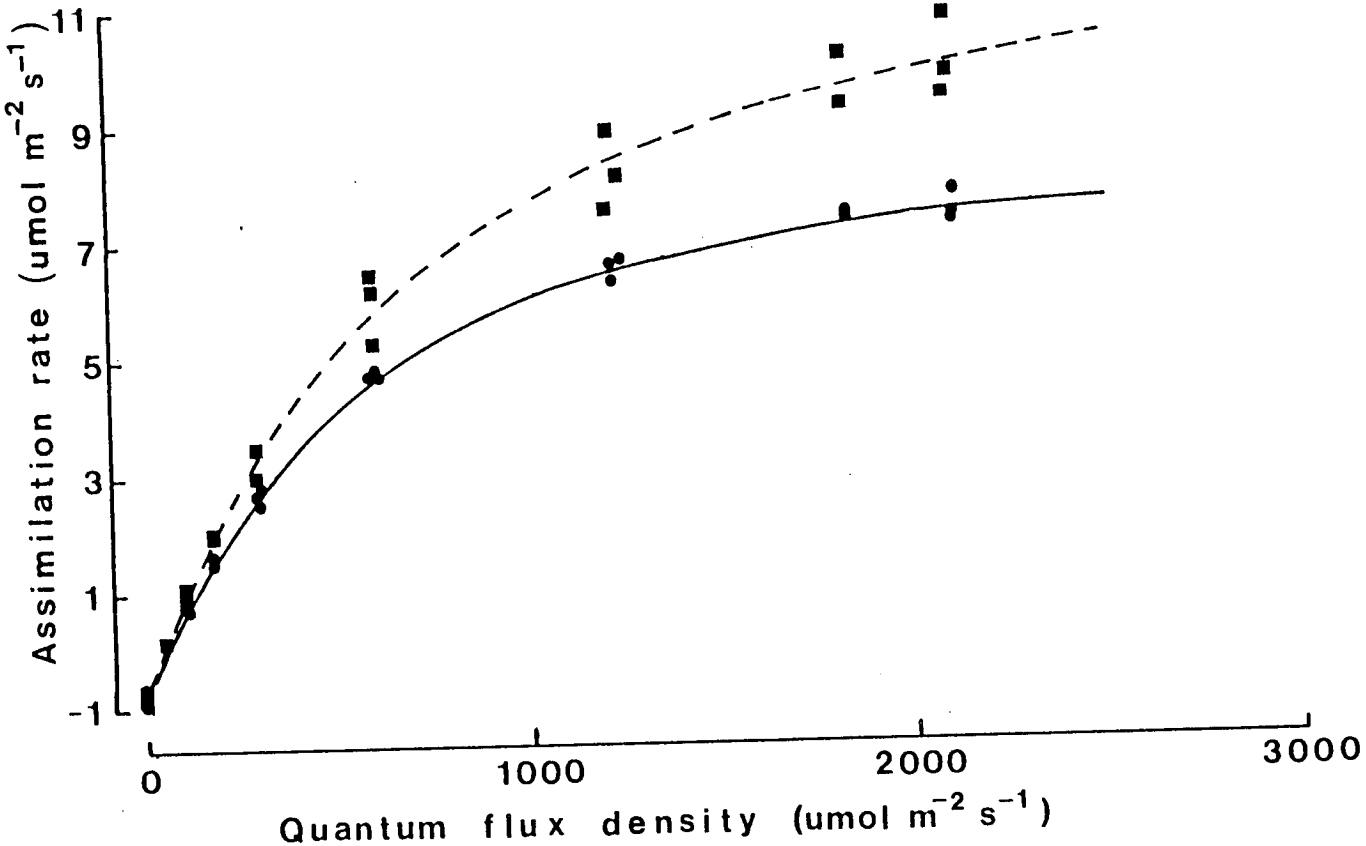


Figure 4.6 Relationship between assimilation rate and quantum flux density for needles on the parent shoot of vegetative (●) and male cone-bearing (■) branches. Fitted lines were drawn using parameters estimated from the model of Jarvis et al. (1985) with a stomatal conductance value of 100 mmol m^{-2} s^{-1}.

Table 4.2 Combined curve analysis of variance tables (Ross 1981) for curves fitted, using the theoretical model of Jarvis etal. (1985) to the CO_2 exchange rates of needles on: (i) parent shoots, (ii) terminal shoots of the current years's growth excluding plant 703, and (iii) including plant 703 of vegetative and male cone-bearing plants of P. contorta.

Curve comparisons	Degree of freedom	Sum of squares	Mean sum of squares	F ratio	P value
(i) parent shoot					
vegetative vs male	4	23.76	5.94	26.59	< 0.001
residual	16	3.57	0.22		
(ii) terminal shoot of the current year's growth excluding plant 703					
vegetative vs male	4	13.22	3.30	1.78	ns
residual	12	22.25	1.85		
(iii)terminal shoot of the current year's growth including plant 703					
vegetative vs male	4	48.12	12.0	3.57	< 0.05
residual	16	53.95	3.37		

Table 4.3 Estimated parameter values from the theoretical model and the maximum assimilation rates (A_{max} μmol m^{-2} s^{-1}) for the needles on: (i) parent shoots and (ii) terminal shoots of the current year's growth of vegetative and male cone-bearing plants of P.contorta (α = initial slope of A/Q curve, g_m = mesophyll conductance (mmol m^{-2} s^{-1}) R_d = dark respiration (μmol m^{-2} s^{-1}), Θ convexity coefficient).

Plant type	α	g_m	Θ	R_d	A_{max}
a) parent shoot					
vegetative	0.0147	31	0.46	0.67	7.29
male	0.0180	46	0.22	0.69	10.29
b) terminal shoot of the current year's growth					
vegetative	0.0167	46	0.10	1.15	9.72
male (701+702)	0.0114	35	0.13	0.74	6.17
male (701+702+703)	0.0082	26	0.35	0.64	3.77

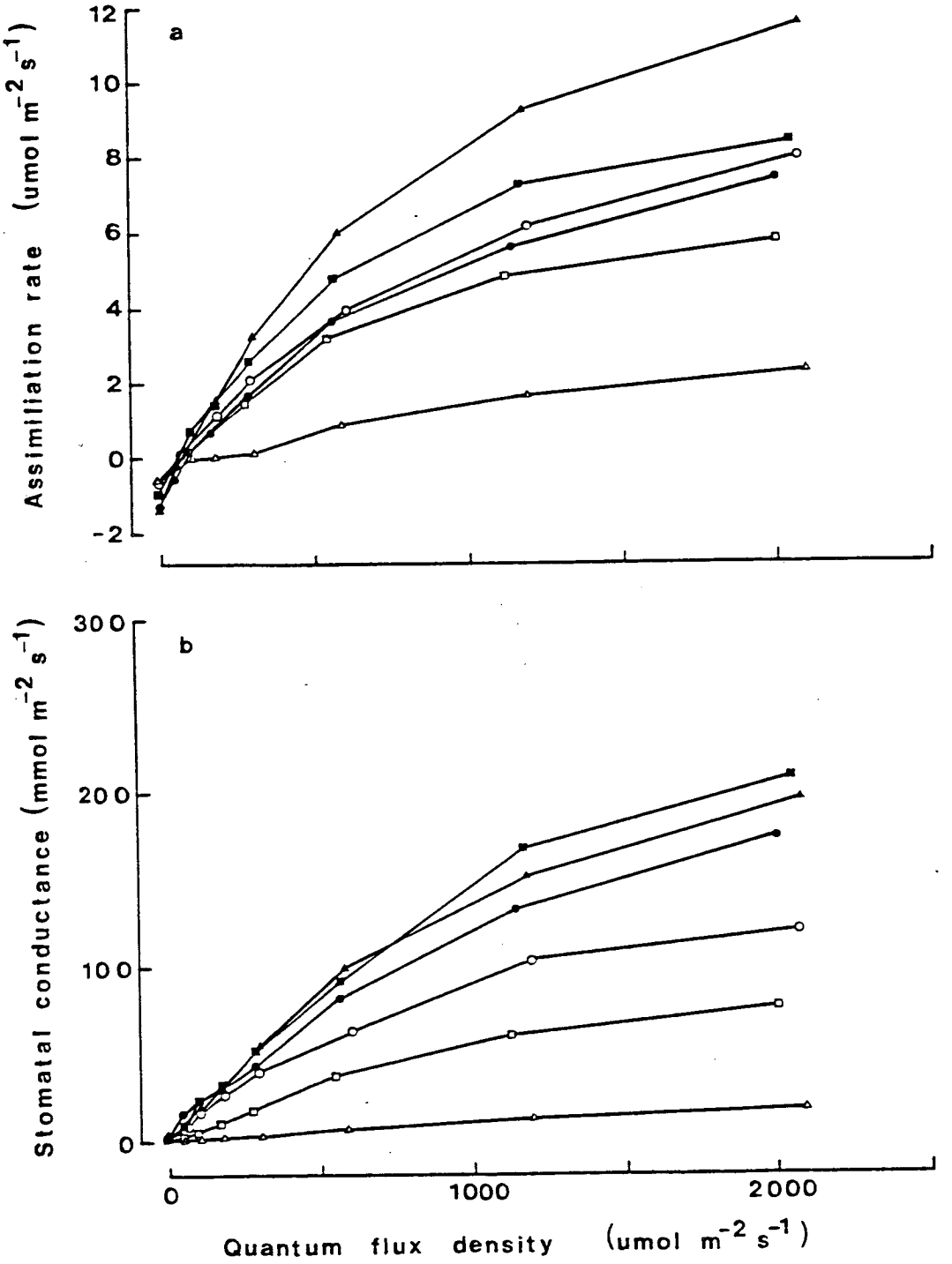


Figure 4.7 The relationship between quantum flux density and a) assimilation rate, b) stomatal conductance for needles on the terminal shoot of the current year's growth of vegetative (601 ● , 602 ■ ,603 ▲) and male cone-bearing (701 ○ ,702 □ ,703 △) plants of P. contorta.

addition the light compensation point was much higher for plant 703 compared with the needles on the other plants (150 μmol m^{-2} s^{-1} compared with 60 - 85 μmol m^{-2} s^{-1}). It is doubtful if plant 703 was a representative sample of the CO_2 exchange rate of needles on the terminal shoot of male cone-bearing plants and should possibly be excluded from the data. If plant 703 was excluded from the data and the model fitted, there was no significant difference between the individual A/Q curves and the common curve fitted to both data sets (Table 4.2). However with plant 703 included there was a small significant ($P < 0.05$) difference between the vegetative and male cone-bearing plants (Figure 4.8).

4.6 DISCUSSION

There was no difference in the dark respiration rate of the parent shoot on vegetative and male cone-bearing plants and the values obtained were very similar to those measured in the field (Chapter 3 Table 3.4). This is not surprising as temperature, the major environmental variable which influences dark respiration, was similar in both studies ($\simeq$ 15 °C).

In contrast, the terminal shoot of the current year's growth of vegetative plants had higher dark respiration rates compared with male cone-bearing plants. There are generally accepted to be two components of respiration; 'growth' and 'maintenance' (see McCree 1978, Penning de Vries 1975). As needle and shoot extension of the current shoot had ceased 2 to 3 months earlier in all plants, it is unlikely that the increased dark respiration rate of vegetative plants was the result of increased 'growth' respiration. 'Maintenance' respiration is generally taken to incorporate the carbon cost of biochemical processes, like the turnover of enzymes and membrane proteins. It is possible that the needles on the vegetative plants had a slightly higher maintenance respiration rate because they had a slightly higher assimilation rate for a given quantum flux density compared with male cone-bearing plants. It is also possible that increases in the dark respiration rates of the current year's shoot were the result of injury caused by removing needles from the stem of the vegetative plants, prior to placing

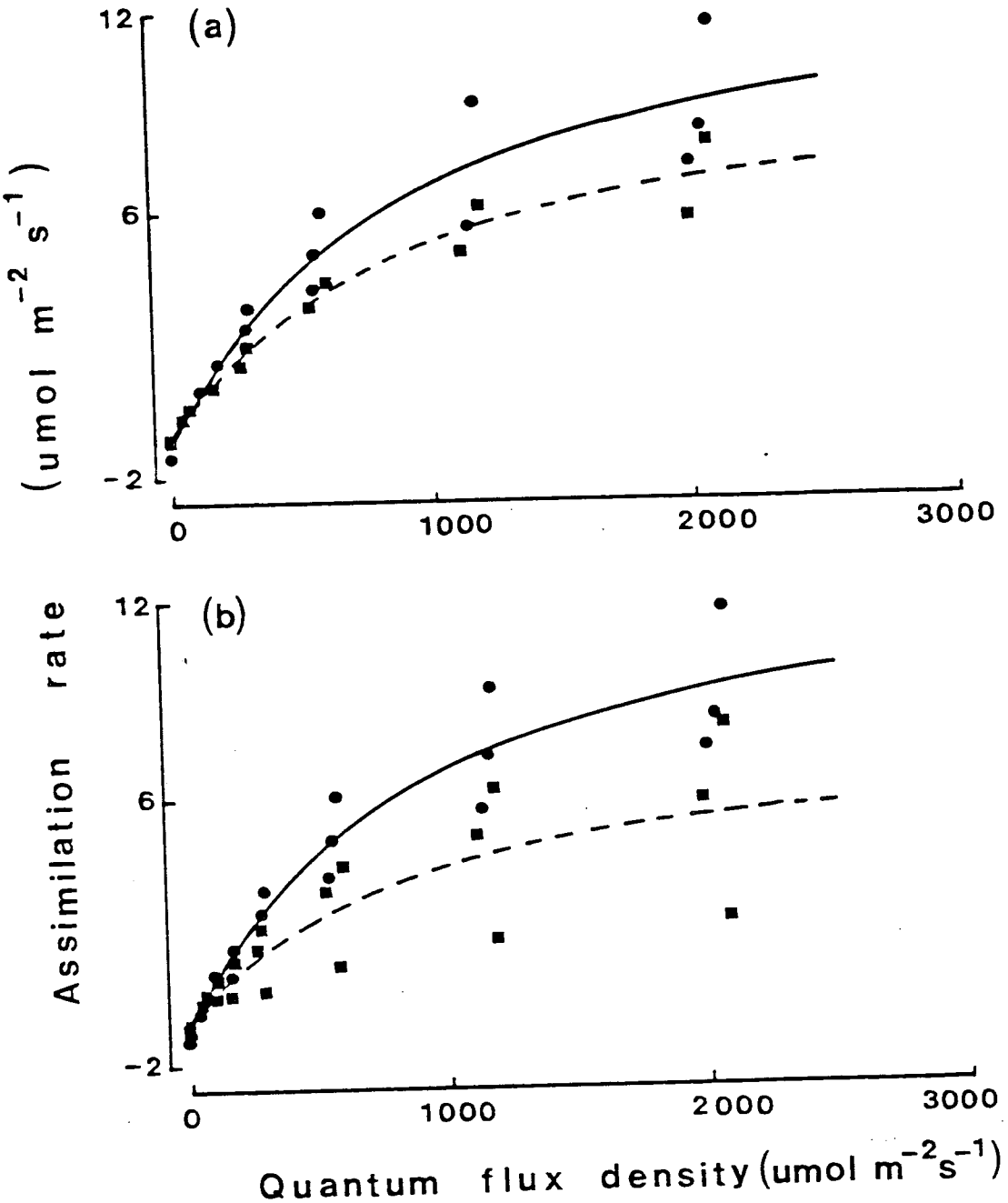


Figure 4.8 Relationship between assimilation and quantum flux density for needles on the terminal shoot of the current year's growth of vegetative (●) and male cone-bearing (■) plants. Fitted lines were drawn using parameters estimated from the model of Jarvis et al. (1985) with stomatal conductance value of 100 mmol m^{-2} s^{-1} , a) with plant 703 excluded and b) with plant 703 included in the analysis.

them in the chamber. Needles were not removed from male cone-bearing plants because the male cones result in a needle free section of stem after the cones have aborted (see Chapter 1 section 1.3.1). Needles had been removed from the current year's shoot of vegetative plants and the parent shoot of vegetative and male cone-bearing plants 5 - 11 days prior to the measurement of CO_2 exchange rates, on the assumption that this would be sufficient time for the plants to recover. It is possible, however, that full recovery may not have been achieved. There was no significant difference between the dark respiration rates of the parent shoots of vegetative and male cone-bearing plants, but needles on the parent shoot had been removed from both sets of plants.

Alternatively, the increase in dark respiration of the current year's shoot on vegetative plants may have been the result of the difference in shoot morphology. The current year's terminal shoot on male cone-bearing plants had an area of stem devoid of needles. It is possible that the respiration rate of that section of stem was reduced because it had fewer vascular connections. It is not possible from this study to determine which of the above was the major factor contributing to the increase in dark respiration of the terminal shoot of the current year's growth on vegetative plants compared with the male cone-bearing plants, although it is possible that they all contributed to some extent.

The needles on the parent shoot of male cone-bearing plants had significantly higher assimilation rates for a given quantum flux density compared with vegetative plants. This confirms the result observed in the field (Chapter 3). It was suggested in Chapter 3 that the increased assimilation rate of male cone-bearing branches was the result of both: (i) increased sink activity in the current male bud, and (ii) reduced source capacity in the current year's terminal shoot because of a reduction in the needle complement. As both vegetative and male cone-bearing plants had male buds in this study, the higher assimilation rate of needles on the parent shoot of male cone-bearing plants was probably the result of a reduced needle complement on the current year's shoot. In addition the needles on the parent shoot of the three male cone-bearing plants

were slightly wider spaced which possible increased their light capturing ability.

The estimated apparent quantum use efficiency (α) was lower in this study compared to the field study for both vegetative and male cone-bearing plants and compared with published values (see Jarvis & Sandford 1986). Although the plants did not appear to be nutrient stressed, it is possible that they were nutrient deficient. They had been potted into larger pots 19 months earlier but had received no additional fertiliser since. Linder & Rook (1984) reviewed the influence of plant nutrient status on the CO_2 exchange of plants and concluded that chlorophyll deficiencies were a common symptom of an unbalanced or inadequate nutrient supply and consequently reduced the photosynthetic efficiency of shoots. The estimated value of A_{max} was similar for male cone-bearing plants in the two studies. However vegetative branches in the field had a higher A_{max} compared to vegetative plants measured in controlled conditions. This was associated with the higher g_m estimated for the plants measured in the field and may also have been caused by poor nutrient supply (Linder & Rook 1984). The higher values of Θ found in this study indicate that there was less self-shading of the needles than in the field (see discussion section 3.6). This was possibly the result of more uniform illumination compared with the field study.

Three replicates were sufficient to determine the difference in the CO_2 exchange rate of needles on the parent shoot of vegetative and male cone-bearing plants. Unfortunately because of the variance between the replicates and the doubtful condition of plant 703 it is not possible from this study to be certain of the difference in the CO_2 exchange rate of needles on the terminal shoot of the current year's growth of vegetative and male cone-bearing plants. As the stomatal conductance was severely limiting in plant 703, it should probably be excluded from the analysis. Unfortunately there was insufficient time to measure the CO_2 exchange rate of another male cone-bearing plant because the measuring system was in great demand. Even although there was probably no significant difference between the assimilation rates of needles on the terminal shoot of the current year's growth of vegetative and male cone-bearing plants,

vegetative plants generally had a higher CO_2 exchange rate for a given quantum flux density. The slight reduction in assimilation rate in male cone-bearing plants may have been caused by the closer packing of the needles possibly resulting in increased self-shading.

The non-significant difference in the CO_2 exchange rate of the terminal shoot of the current year's growth may be explained if it is assumed that the male bud on both sets of plants was controlling the assimilation rate of the current year's needles. Bidwell (1983) concluded that there was substantial evidence that plant growth regulators originating in active sink organs controlled the photosynthetic rate of leaves. He cited several worker who have found that exogenously applied indole acetic acid and gibberellic acid induce higher assimilation rates in a variety of plants. Both these plant growth regulators are associated with the induction, growth and development of cones on conifers (see reviews by Lee 1979, Ross & Pharis 1985).

In conclusion, it would appear that the needles on the parent shoot of male cone-bearing plants have higher assimilation rates as a result of a reduction in the needle complement of the current year's shoot because of the earlier presence of male cones. Unfortunately, the influence of this reduction in needle numbers could not be assessed independently from the possible influence of growing male buds which were present on both vegetative and male cone-bearing plants. It has been found (see Ericsson 1980) that in the autumn assimilates produced in needles on the parent and current year's shoot are primarily exported to the stem and roots. In addition, assimilates produced by needles on the current year's shoots are also exported to the growing bud. If stem and root growth are not to be suppressed in male cone-bearing plants, it follows that needles on either the parent and/or the current year's shoot would need to assimilate at a higher rate than equivalent vegetative plants. It has been shown in both field and controlled conditions that needles on the parent shoot of male cone-bearing branches do indeed have a higher assimilation rate compared to equivalent needles on vegetative plants. This would tend to indicate that growth of stem wood and roots may not be suppressed in male

cone-bearing plants. In order to determine the influence of cones on the overall growth of plants a potted plant system was devised in which the influence of male, n1 and n2 female cones could be assessed independently and this is discussed in Chapter 6.

4.7 SUMMARY

1) Needles on the terminal shoot of the current year's growth of vegetative plants had higher dark respiration rates than equivalent needles on male cone-bearing plants. However there was no significant difference between the dark respiration rate of needles on the parent shoot of vegetative and male cone-bearing plants.

2) The increase in the dark respiration rates of the current year's needles of vegetative plants may have be the result of a combination of the following: (i) increased maintenance respiration, (ii) injury caused by removing needles from the shoots on vegetative plants, (iii) reduced stem respiration on male cone-bearing plants caused by a reduction in the number of vascular connections resulting from the abcission of the male cones.

3) The needles on the parent shoots of male cones-bearing plants had significantly higher assimialtion rates for a given quantum flux density compared to equivalent needles on vegetative plants. This confirms the results from measurements in the field (Chapter 3).

4) The increase in assimialtion rates of the needles on the parent shoot of male cones-bearing plants is probably the result of fewer current year's needles.

5) It is not possible from this study to determine the difference in the assimilation rates of needles on the terminal shoot of vegetative and male cone-bearing plants, because of variation in the data. It would appear, however, that there was no significant difference between the assimialtion rates of needles on the terminal shoot of the current year's growth of vegetative and male cone-bearing plants. This may be because both vegetative and male cone-bearing plants had current male buds.

CHAPTER 5

THE RESPIRATION RATE OF REPRODUCTIVE STRUCTURES AND VEGETATIVE BUDS

5.1 AIM

Reproductive structures of many plants have a reduced rate of net respiration when exposed to light. It was the aim of this study to to quantify the reduction in net respiration rates of female cones of P. contorta, and determine if male buds and vegetative buds also exhibit this phenomenon.

5.2 INTRODUCTION

It has been shown that reproductive structures possess a system which refixes CO_2 from mitochondrial respiration (see review by Blake & Lenz 1989). On fruit trees, it has been estimated that the young green fruit refix sufficient CO_2 to conserve 25 - 80% of the CO_2 released by dark respiration (see review by Schaedle 1975). Bazzaz et al. (1979) have determined the contribution of fruit and flower photosynthesis to the total carbon required for production of mature seed in 15 temperate, amenity deciduous trees. They showed that the percentage contribution of in situ photosynthesis to the total carbon balance of the fruit or flower ranged from 64.5% for Acer platanoides to 2.3% for Quercus macrocarpa. Similar reductions in the rate of dark respiration of female cones in coniferous trees have been demonstrated, rangeing from 0% - 79% depending on the stage of development of the cone (Rook & Sweet 1971, Linder & Troeng 1981, Koppel et al. 1987). As male cones are green under the scales they may also be capable of reduced respiration in the light, (i.e. refixation) no studies indicating this could be found in the literature.

Male cones are initiated in early to mid-summer (Owens & Molder 1975, Couper 1987). They are enclosed in bud scales until early the following spring, as described in Chapter 1 (section 1.3.1). The male cones are normally formed in the basal section of a bud with needle primordia in the apical region. It is, therefore, very

difficult to estimate the refixation of CO_2 by male cones separately from that of the needle primordia which are also enclosed within the same bud. For this reason refixation by male cones was estimated by comparing the refixation capacity of male cone-bearing buds, comprising of needle primordia and male cones with vegetative buds which contained only needle primordia.

Female cones are also initiated in the summer but they are enclosed in their own bud scales until the following spring. They then emerge and remain on the tree for a further 18 - 20 months. Because they are isolated in this way female cones were easier to investigate. In addition, as they can be detached from the tree and their respiration rates measured independently from those of other structures.

5.3 MATERIALS

The respiration rates of female cones and male cone-bearing buds could not be measured throughout their whole life cycle (i.e. from initiation to abscission) because it was not possible to identify the reproductive structures until several months after initiation. In addition, female cones are extremely susceptible to desiccation prior to pollination. Therefore, it was planned to measure the respiration rate of:
(i) female cones starting as soon after pollination as possible,
(ii) male cone-bearing buds as soon as they could be identified, and
(iii) vegetative buds at the same time as the male cone-bearing buds.

The equipment used to measure these respiration rates was not available, however, until April 1987. It was therefore decided to start measuring the respiration rates of female cones, male cone-bearing buds and vegetative buds from this date and to continue to the end of March 1988. In this way all stages of the life cycle were sampled although not in the same reproductive cycle. To simplify the presentation of results in this chapter the data are shown as if they were all collected in the same reproductive cycle. The assessment dates are expressed as weeks from the estimated date

of female cone pollination and male cone initiation, which was taken to be the 1st of June (Figure 5.1).

Female cones were collected from two genotypes during the sampling period because sampling started in April and the n2 female cones were removed with the whole of the previous year's and current year's shoots including the n1 female cones. Therefore it was not possible to assess the respiration rates of both n1 and n2 female cones from the same genotype. The n2 female cones were sampled from early April 1987 until they senesced in September 1987, while n1 female cones were sampled from late August 1987 through to late March 1988.

Female cones initiated in 1985 (n2) were collected from a group of three P. contorta trees of clone 13, (Skagway provenance). These trees had been collected and rooted as cuttings in 1972 (from trees growing at Sela Muir Forest, Scotland), and where planted in 1973 at the I.T.E. 'Farfield' plot, near Roslin, Midlothian, Scotland (3° 10' longitude, 55° 50' latitude, 184 m altitude). Female cones initiated in 1986 were sampled from a seedling of Cedarvale, (Skeena River, B.C. provenance) planted in 1971, at the I.T.E. 'Nursery' plot, Penicuik, Midlothian, Scotland, when one year old.

The number of female cones sampled at each assessment date varied depending on the size of the cones (Table 5.1). The CO_2 exchange of three replicates were measured at each assessment date.

Male and vegetative plants were selected from the same material as used in Chapter 4. The cuttings were struck in February 1985 and potted in May 1985. The parent trees were also rooted cuttings from Shin, near Lairg, Scotland, growing in the nursery of the Forestry Commission, Roslin, Scotland. The original seed came from the south coast of Washington State, USA. The respiration rates of male cone-bearing and vegetative buds were measured from early April to the end of May 1987. Young buds were also collected in early October 1987, but the respiration rates could not be measured accurately because the CO_2 exchange rate of the buds was very low and it was not possible to insert more than six buds at a time into

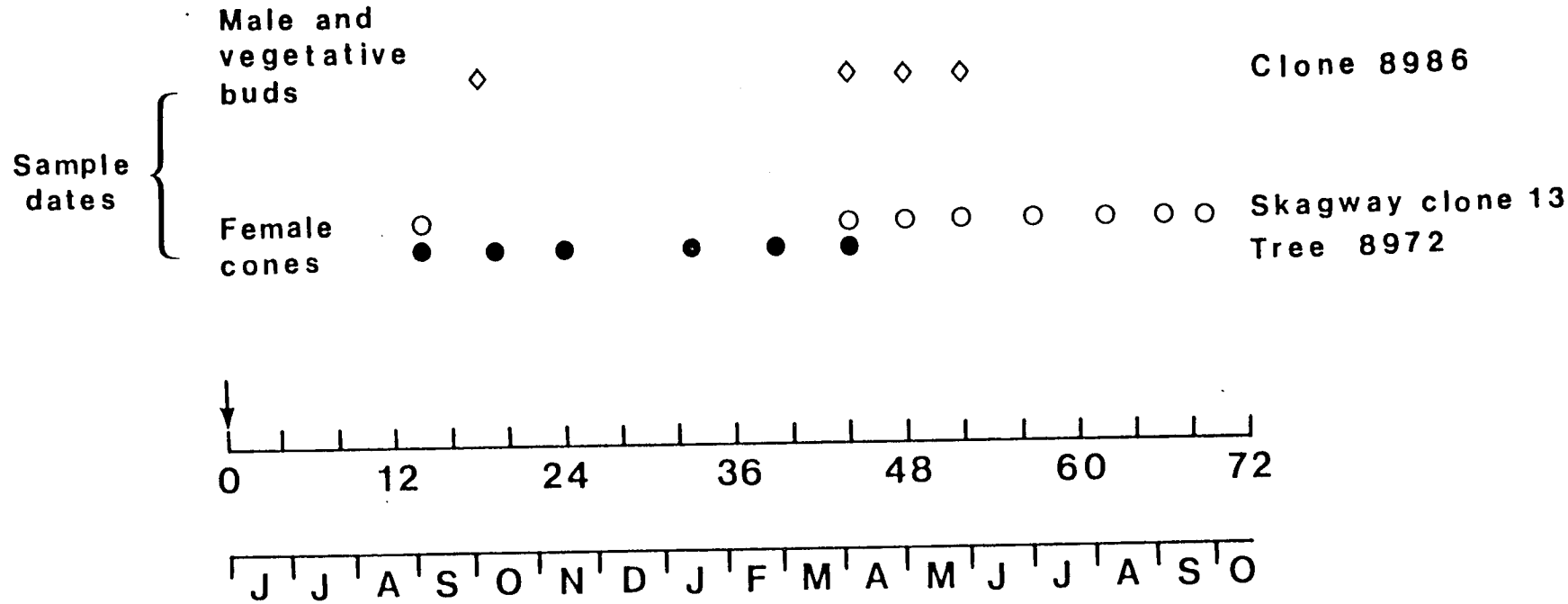


Figure 5.1 Assessment dates of the respiration rates of female cones, vegetative and male cone-bearing buds of P. contorta sampled and the weeks from the estimated date of female cone pollination and male cone initiation (1st June).

the assimilation chamber and obtain a good seal. The number of buds sampled at each assessment varied depending on the size of the buds (Table 5.2). The CO_2 exchange of three replicates were measured at each assessment date.

Table 5.1 Assessment dates, weeks from the estimated date of female cone pollination (1st June), the number of female cones of P. contorta assessed in the chamber at each sample date and the mean projected projected area of one surface of the cone (one standard error of the mean).

week from 1st June	Date	no. of cones per replicate	projected area of a cone (cm^2)
14	29-31/8/87	5	1.2 (0.14)
19	5-9/10/87	18	1.5 (0.03)
24	12-14/11/87	18	1.4 (0.08)
33	9-11/1/88	12	1.5 (0.08)
39	24-27/2/88	10	1.6 (0.08)
44	3-5/4/87	4	1.5 (0.03)
48	28/4-1/5/87	4	2.8 (0.10)
52	29-31/5/87	1	7.0 (0.37)
57	27-28/6/87	1	7.6 (0.21)
62	31/7-3/8/87	3	6.8 (0.31)
66	29-31/8/87	3	6.1 (0.24)
69	24-25/9/87	4	6.7 (0.28)

Table 5.2 Assessment dates, weeks from the estimated date of male cone initiation (1st June), the number and mean projected area of one surface of vegetative (veg) and male buds of P. contorta assessed in the chamber at each sample date (one standard error of the mean).

Week	Date	no. buds per replicate		projected area (cm^2)	
		veg	male	veg	male
18	2-3/10/87	6	6	1.1 (0.08)	1.6 (0.08)
44	30/3-5/4/87	3	3	2.2 (0.15)	2.6 (0.19)
48	27/4-1/5/87	3	2	3.3 (0.32)	5.2 (0.61)
52	26-29/5/87	2	1	5.5 (0.56)	10.2 (0.80)

5.4 METHOD

Branches bearing female cones were cut at the base of the previous year's shoot and taken directly to the laboratory in a moist polythene bag. The cones were detached from the branch, and the cut end of the cone was sealed with Lanolin (BDH Chemicals Ltd. Poole, England). In contrast, vegetative and male cone-bearing buds were placed in the chamber while still attached to the plant. The projected area and weight of the cones or buds were measured when they were removed from the assimilation chamber. The surface area (Table 5.1 & 5.2) was measured using a leaf area meter (LI 3100, LiCor Inc., Lincoln, Nebraska, USA). The total dry weight of the cones or buds in the chamber was measured on an electronic balance (Sauter RE 1614, August-Sater-Strabe, Postfach, Switzerland).

The respiration rates of the cones and buds were measured in the photosynthesis laboratory described in Chapter 4. The leaf chamber was bilaterally illuminated with metal-halide lamps (HQI, 400 W, Wontan Ltd., London, England). The light emitted from each lamp was collimated by aspheric Fresnel lens made from acrylic plastic with a focal length 31.8 cm (Ealing Electro-optic plc, Watford England). The quantum flux density in the chamber was controlled with neutral density filters (Frew-Smith Ltd., Irvine, Scotland). The following conditions were controlled within the assimilation chamber; cone or bud temperature at 15 °C, ambient CO_2 concentration at 350 μmol mol^{-1}, water vapour pressure difference across the cone or bud surface at 0.8 kPa. The cones or buds were carefully placed in the assimilation chamber so that they did not shade each other. They were left for 30 to 75 minutes in the dark to equilibrate before the lights were switched on. To increase the quantum flux density within the chamber the neutral density filters were changed, when the respiration rate had been constant for 15 minutes.

The percentage refixation capacity (ω) of the cones and buds was calculated following Linder & Troeng (1981) :

$$\omega(Q) = 100 \cdot (R(0) - R(Q) / R(0)) \qquad (5.1)$$

where R(0) is the respiration rate of the cone or bud at zero quantum flux density, and R(Q) is the respiration rate of the cone or bud when illuminated.

The net respiration rate of a female cone throughout it's life was modelled assuming that:
(i) the respiration rate of a cone is primarily dependent on temperature, quantum flux density and the age of the cone, and
(ii) the respiration rate of a cone increases exponentially with temperature, and decreases exponentially with quantum flux density. This occurs independently, in a similar manner to that proposed by Reed et al. (1976) for leaves.

The equation for the response of cone or bud respiration rate to temperature, at a particular time of year is :

$$R_d(T) = R_d(0) \cdot \exp(\eta T) \qquad (5.2)$$

where R_d is the dark respiration rate of a cone (nmol g^{-1} s^{-1}),
$R_d(0)$ is R in the dark at 0 °C (nmol g^{-1} s^{-1}),
T is temperature (°C), and
η is the temperature coefficient.

The equation for the response of cone respiration to quantum flux density, at a particular temperature and time of year is:

$$R_n(Q) = R(Q_{max}) + (R(Q_0) - R(Q_{max})) \cdot \exp(-\beta Q) \qquad (5.3)$$

where R_n is the net respiration rate of a cone (nmol g^{-1} s^{-1}),
Q is quantum flux density (μmol m^{-2} s^{-1}),
R(Q) is the respiration rate of cones at a given quantum flux density, $Q=Q_{max}$ is light saturation and $Q=Q_0$ is darkness, and
β is the quantum flux coefficient. Clearly, $RQ_0 = R_d$ at a particular temperature.

The initial slope (α) of the respiration quantum response curve for female cones at a particular temperature is the apparent quantum use efficiency of the female cone and is calculated as:

$$\alpha = -\beta\ (R(Q_0) - R(Q_{max}). \qquad (5.4)$$

Combining equations 5.2 and 5.3 gives a function which estimates the net respiration rates (i.e. the CO_2 efflux) of cones for a particular time of year.

$$R_n(T,Q) = R(T,Q_{max}) + \left[(R(0,0)\ .\ \exp(\eta T) - R(T,Q_{max})\right] \exp(-\beta Q). \qquad (5.5)$$

The input variables are:
(i) quantum flux density (μmol m^{-2} s^{-1}),
(ii) temperature (°C),
(iii) the temperature coefficient (η),
(iv) the quantum flux coefficient (β),
(v) the respiration rate of cones in the dark at 0 °C (nmol g^{-1} s^{-1}), and
(vi) the respiration rate of cones at light saturation (nmol g^{-1} s^{-1}).

The values of β and $R(Q_{max})$ were estimated by fitting an exponential curve (Genstat version 5) to the light response data at 15 °C collected at intervals throughout the year. Fourth order polynomials were then fitted (SAS) to the values of β and $R(Q_{max})$ to estimate these variables throughout the year.

As a constant temperature of 15 °C was used in this study, η could not be estimated from the data collected. The exponential function found by Linder & Troeng (1981) for the respiration rates of female cones of Pinus sylvestris in the dark, was utilised in the model:

$$R_d(T,t) = 0.354\ .\ R_d(15,t)\ .\ \exp(0.0693T) \qquad (5.6)$$

where $R_d(15)$ is the respiration rate of female cones at 15 °C in the dark and t is the time of year.

$R_d(15,t)$ was estimated throughout the year by fitting a sixth order polynomial (using SAS) to the sequence of measured $R_d(15)$ values.

The dry weight of the female cone was estimated throughout the year by fitting a logistic curve to the dry weight of the female cones collected.

Mean hourly values of Q and T for the period September 1987 to September 1988 were obtained from Dr. A. Crossley and Miss D. Wilson of I.T.E. The data were collected using a weather station (Precision Model, Didcot Instruments Company Ltd. Abingdon, England) at Glentress forest, Peeblesshire Scotland (3° 8′ longitude, 55° 39′ latitude, 275 m altitude (see I.T.E. contract report C.E.C. T07004a1). The quantum flux density was reduced by 25% to give a reasonable estimate of the light conditions at the level where most of the cones would be found in the canopy. A reduction factor of 25% was used by Linder & Troeng (1981) and was also estimated by 'MAESTRO' (see Chapter 7).

5.5 RESULTS

5.5.1 FEMALE CONES

The cones began to increase in surface area and weight in early spring following a period of winter dormancy (Table 5.1 & Figure 5.2). The period of maximum growth was between week 48 and week 52. The surface area increased by 250% during this period, while the fresh weight increased by 150%. The cones had stopped increasing in surface area by week 52 and in dry weight by week 62.

The dark respiration rate of the female cones fall into 3 phases:
(i) a period of relatively low respiration rate during the winter,
(ii) a period of high respiration rate during spring and early summer and
(iii) a period of decreasing respiration rate from mid-summer until the cones senescence in the autumn (Figure 5.3).

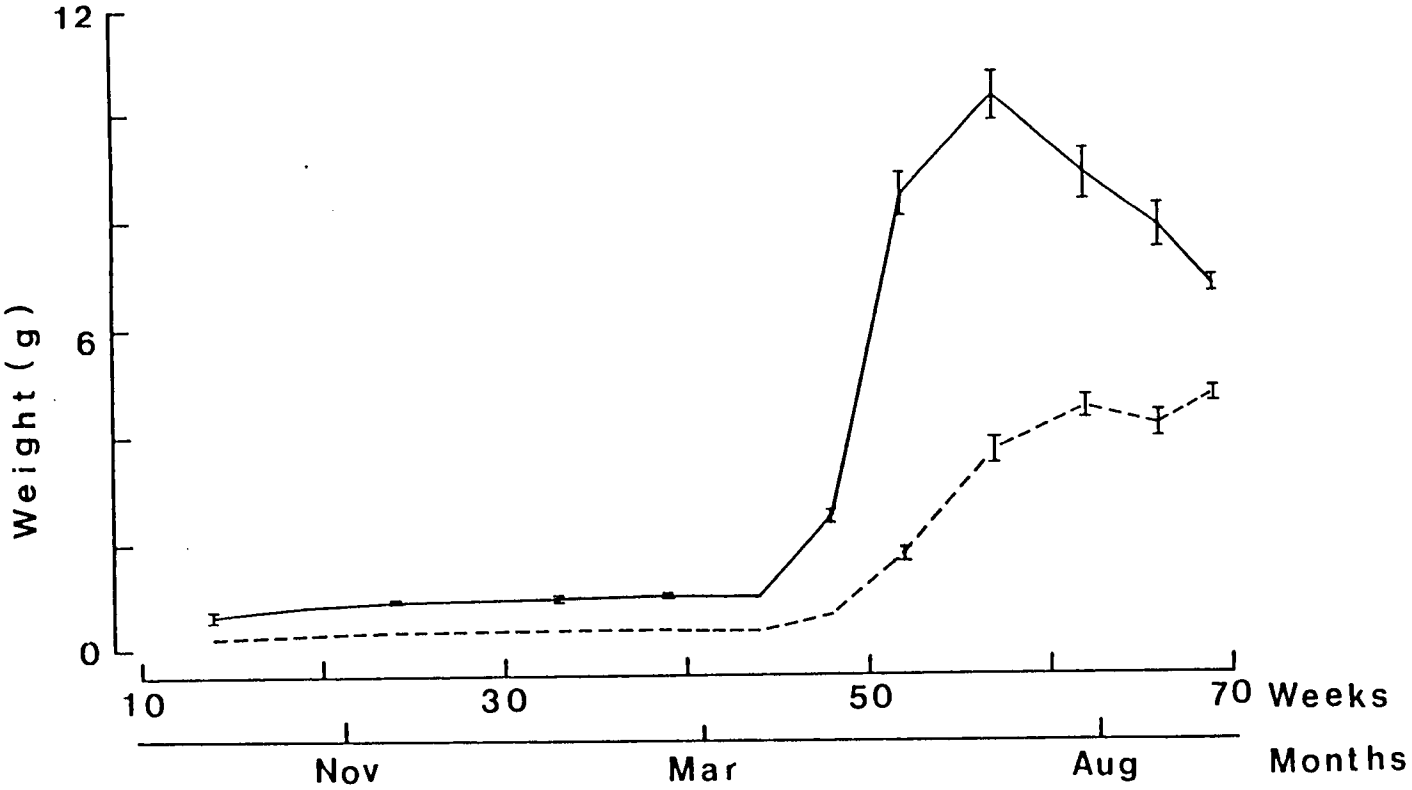


Figure 5.2 Mean fresh weight and dry weight of P. contorta female cones (± one standard error of the mean).

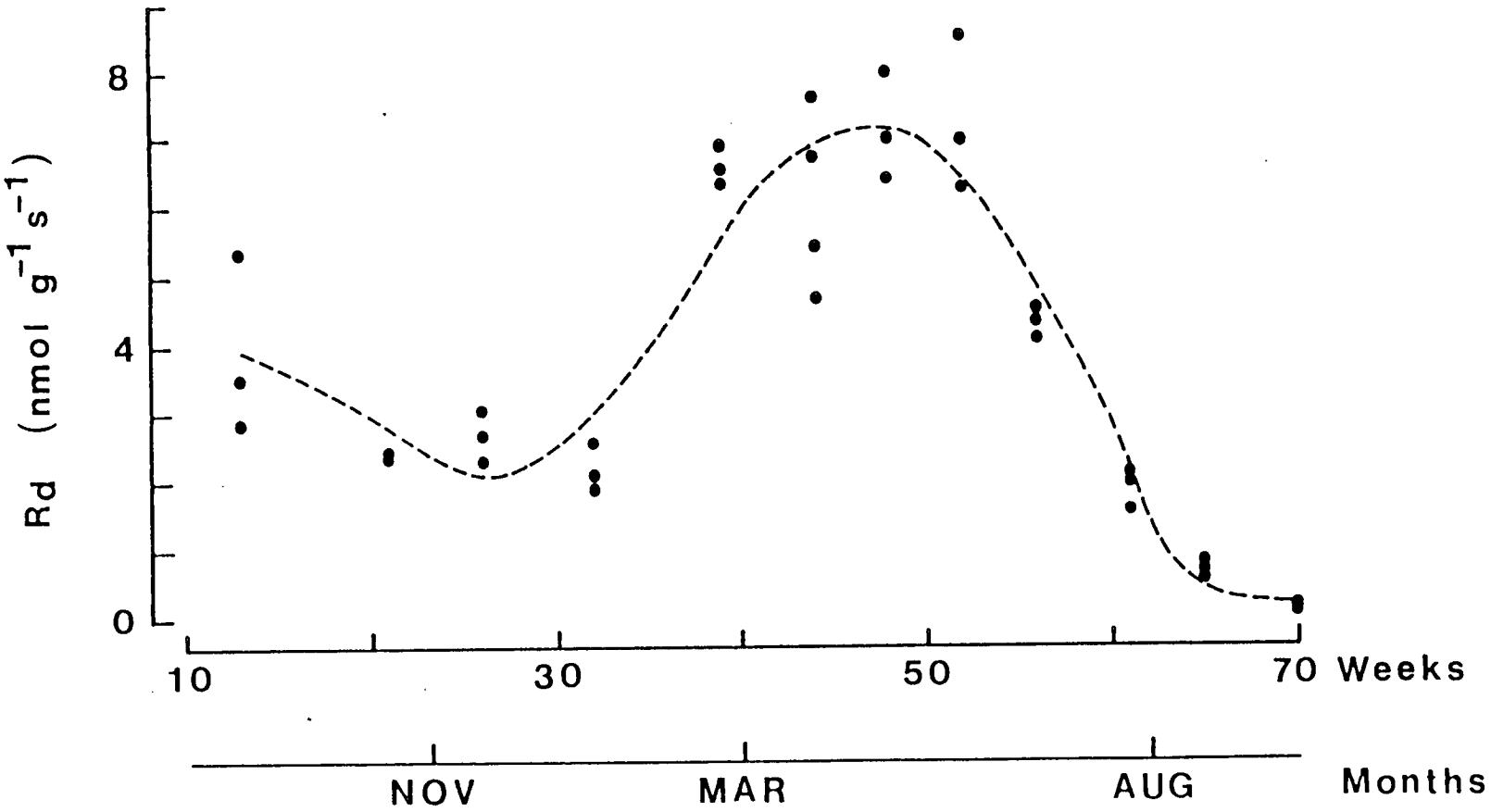


Figure 5.3 Dark respiration rate of P. contorta female cones (---- 6th order polynomial).

The response of the female cones to quantum flux density also occurred in three phases (Figure 5.4). The initial slope of the light response curve (i.e. the quantum use efficiency, α) was largest in weeks 48 and 52 (Table 5.3). Female cones had very low respiration rates when exposed to quantum flux densities higher than 1500 μmol m^{-2} s^{-1} during the winter and early spring (Figure 5.4). The percentage refixation capacity was close to 100% during the winter and generally declined during the summer, falling to zero by the autumn (Figure 5.5).

Table 5.3 Estimated parameters from fitting exponential curves to the relation between respiration rates at 15°C and the quantum flux density for female cones of P. contorta throughout the life of the cone. Weeks numbered from the estimated date of pollination, 1st June. ($R(Q_{max})$ = respiration rate of female cones at light saturation (nmol g^{-1} s^{-1}), α = initial slope of the R/Q curve, β light coefficient, $R(Q_0)$ = dark respiration rate (nmol g^{-1} s^{-1}).

Week	$R(Q_{max})$	α	β	$R(Q_0)$	% variance accounted
14	0.050	10.82	3.36	3.27	97.2
19	-0.026	4.82	1.85	2.63	96.0
24	0.025	5.00	1.84	2.69	95.6
33	-0.293	3.17	1.27	2.20	94.0
39	0.019	7.41	1.10	6.75	97.7
44	0.925	7.36	1.57	5.61	81.6
48	1.176	12.81	2.16	7.11	95.3
52	1.292	15.07	2.54	7.22	92.9
57	1.442	5.14	1.77	4.35	98.6
62	0.772	2.77	2.56	1.85	83.3
69	0.326	0.11	3.10	0.69	69.5

A fourth order polynomial gave a good estimation of β (Figure 5.6) and $R(Q_{max})$ (Figure 5.7).

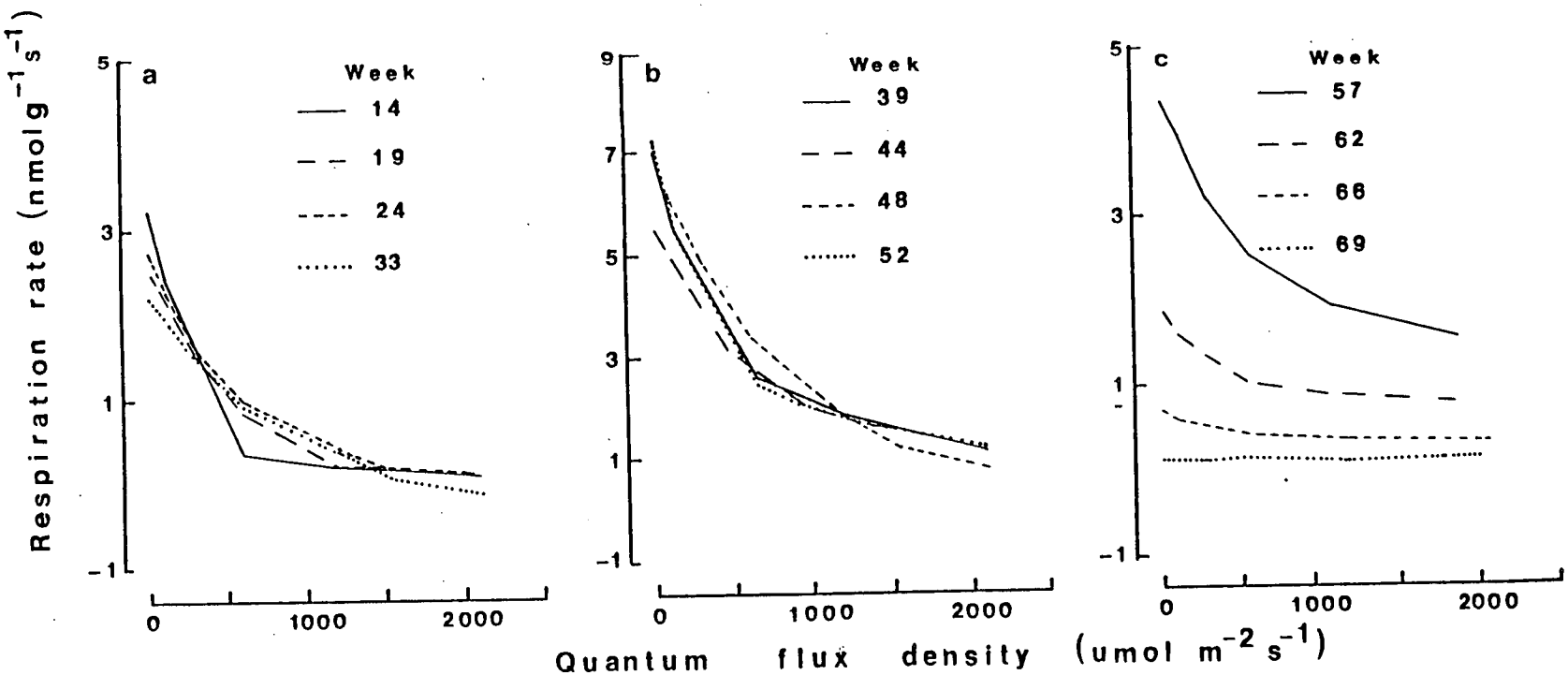


Figure 5.4 The relationship between respiration rate of female cone of P. contorta and incident quantum flux density. Weeks are counted from the 1st June, the estimated date of pollination.

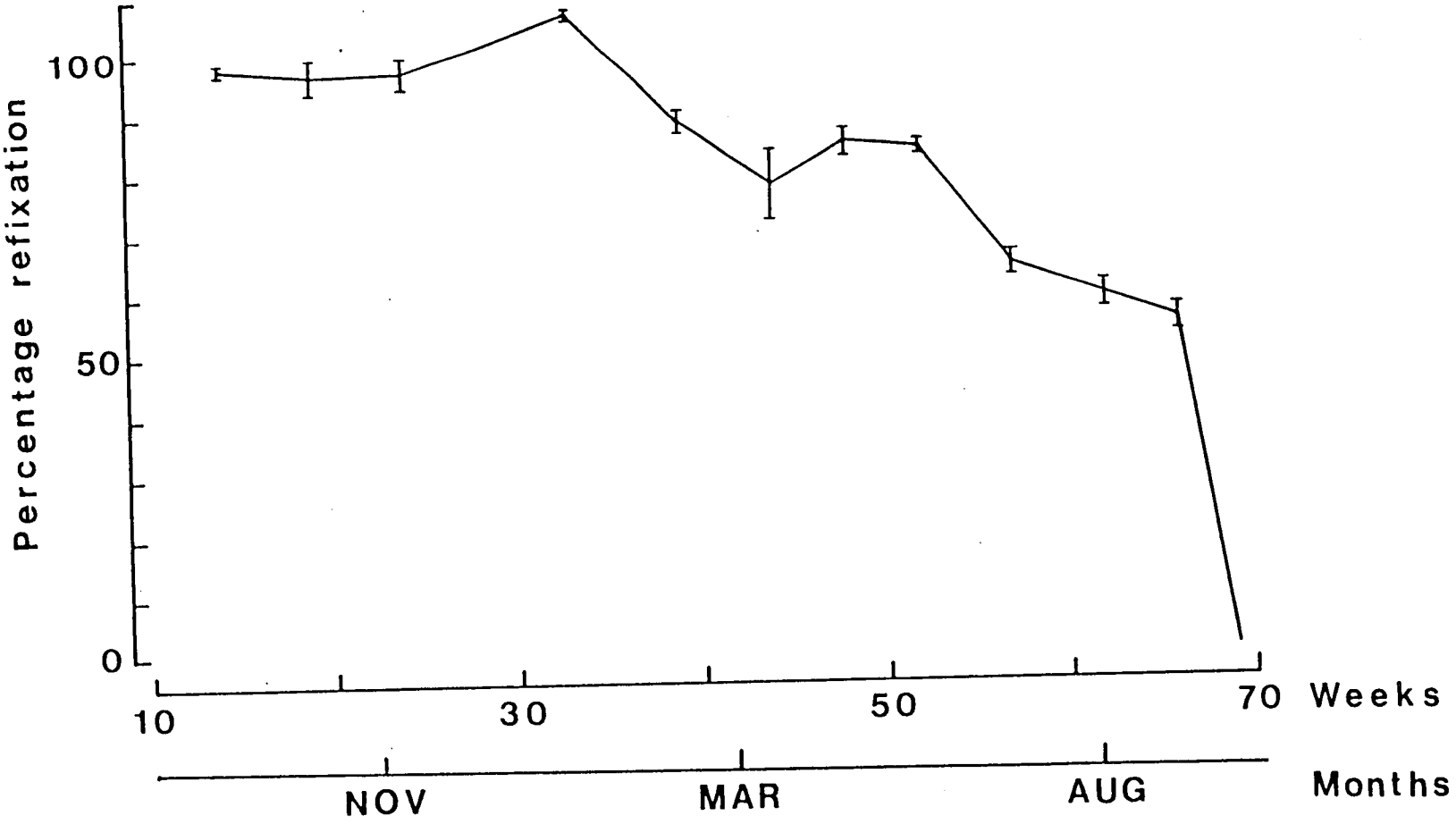


Figure 5.5 Refixation capacity of P. contorta female cones at quantum flux densities higher than 1500 umol m^{-2} s^{-1}.

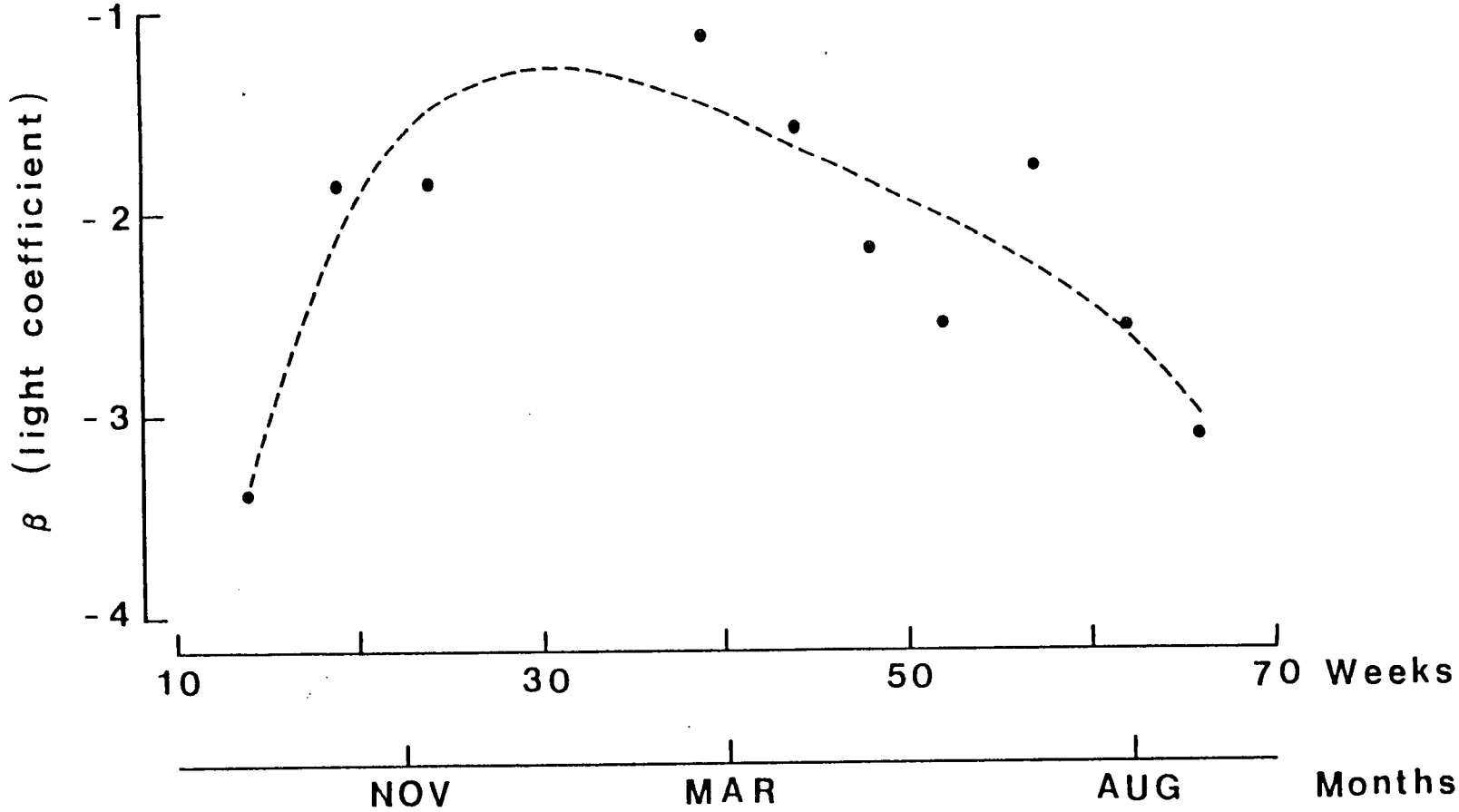


Figure 5.6 Fourth order polynomial fitted to the estimated values of the light coefficient (see text) for female cones of P. contorta.

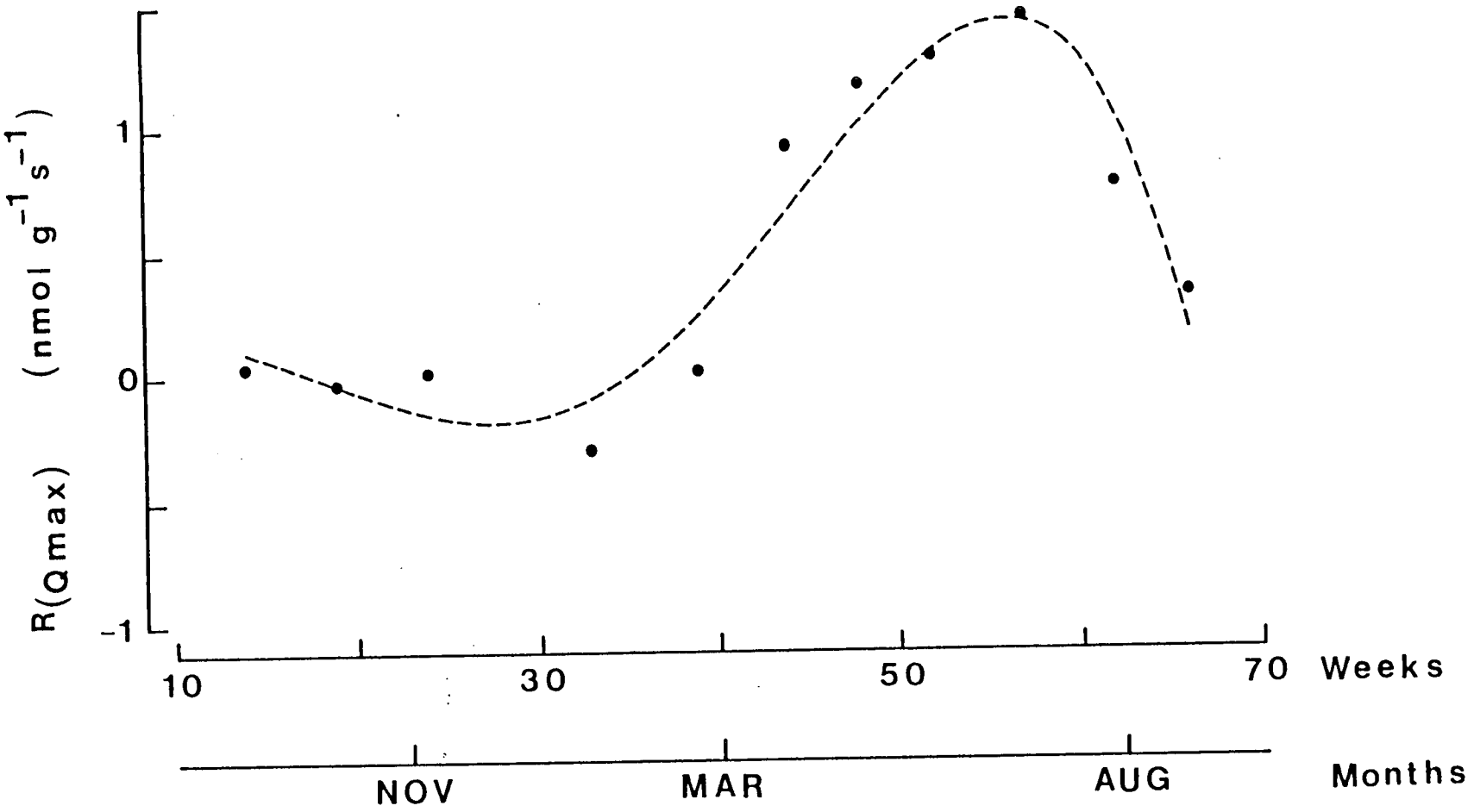


Figure 5.7 Fourth order polynomial fitted to the estimated values of $R_{(Qmax)}$ (the repiration rate at maximum quantum flux density) of P. contorta female cones.

Both environmental variables (Q and T) showed normal seasonal trends of low values in winter and high in summer (Figure 5.8). The estimated net and dark respiration rates of female cones from September 1987 to September 1988 (Figure 5.9) shows the interaction between environmental variables and the physiological efficiency of female cones. The period of maximum refixation capacity by the female cones was during the winter (Figure 5.5). However the model predicts a very low refixation percentage (mean of 4.5%) for November to February. The low refixation percentage reflects the low values of Q and T during the winter. During the early spring and summer substantial refixation occurred, peaking in June with an average of 27.5% (Figure 5.10). Thereafter the refixation and respiration rates of the cones declined towards the autumn.

The total estimated net respiration from week 14 to week 70 was 2.6 g CO_2 $cone^{-1}$ (i.e. 0.71 g carbon). Assuming a 50% carbon content of the cone (Linder & Troeng 1981), the carbon demand for the development of one cone (4.5 g) would be: cone biomass 2.25 + net respiration 0.71 = 2.96 g carbon. Therefore the cost of producing one cone in terms of net respiration was 31%. The total estimated dark respiration from the same period (September 1987 to September 1988) was 3.2 g CO_2 (0.94 g C). Therefore the average refixation capacity was 25%.

5.5.2 MALE CONES

Compared with vegetative buds, male cone-bearing buds had a larger surface area (Table 5.2) and dry weight (Figure 5.11) 18 weeks after the estimated date of male cone initiation, i.e. 1st June. By week 52, male cone-bearing buds had 400% more dry weight and a 100% more surface area than vegetative buds. The male cone-bearing section of the terminal buds accounted for between 65-80% of the tissue present in the assimilation chamber (Table 5.4).

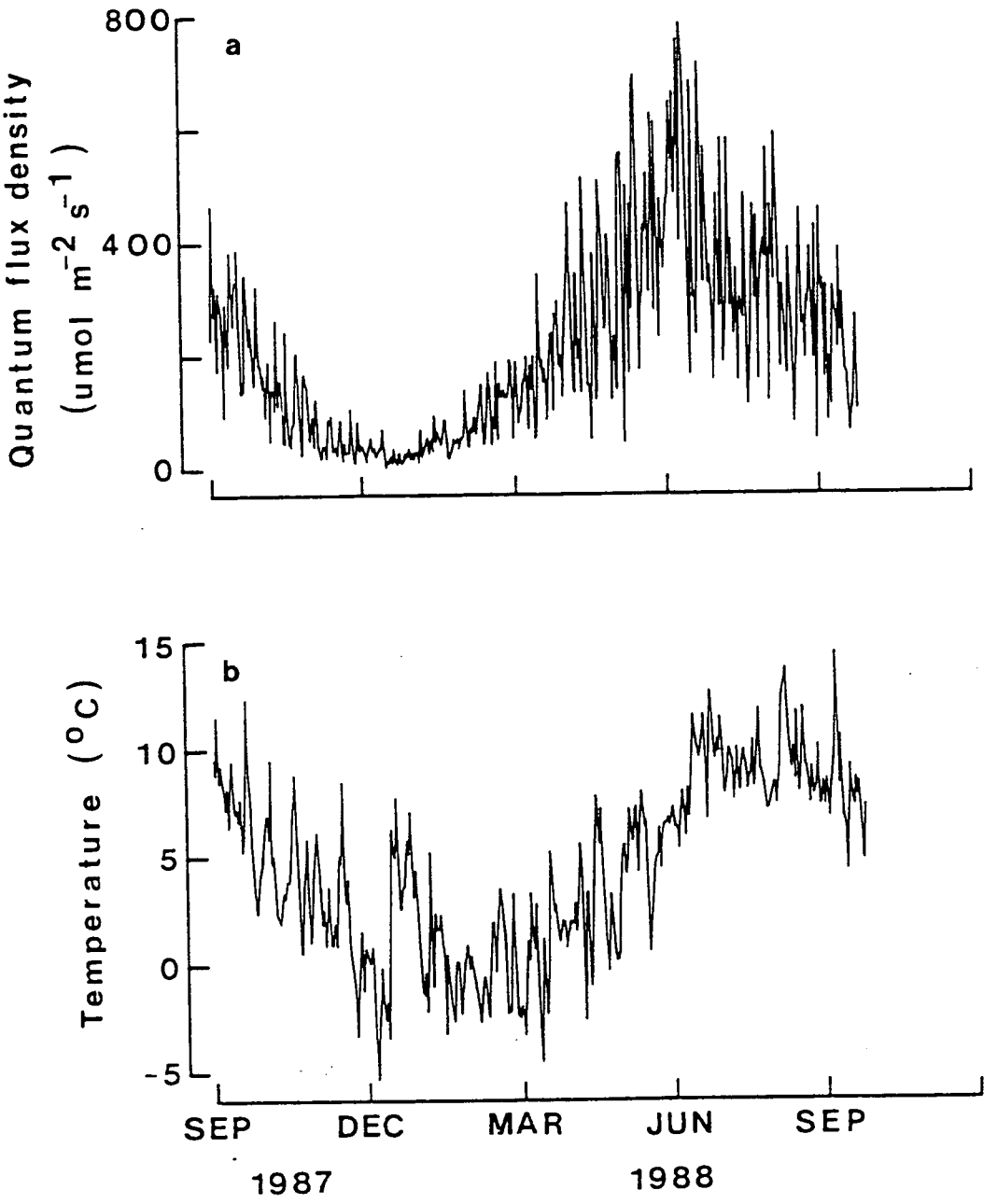


Figure 5.8 a) Quantum flux density and b) temperature from September 1987 to September 1988 at Glentress Forest, Peebles, Peeblesshire, Scotland.

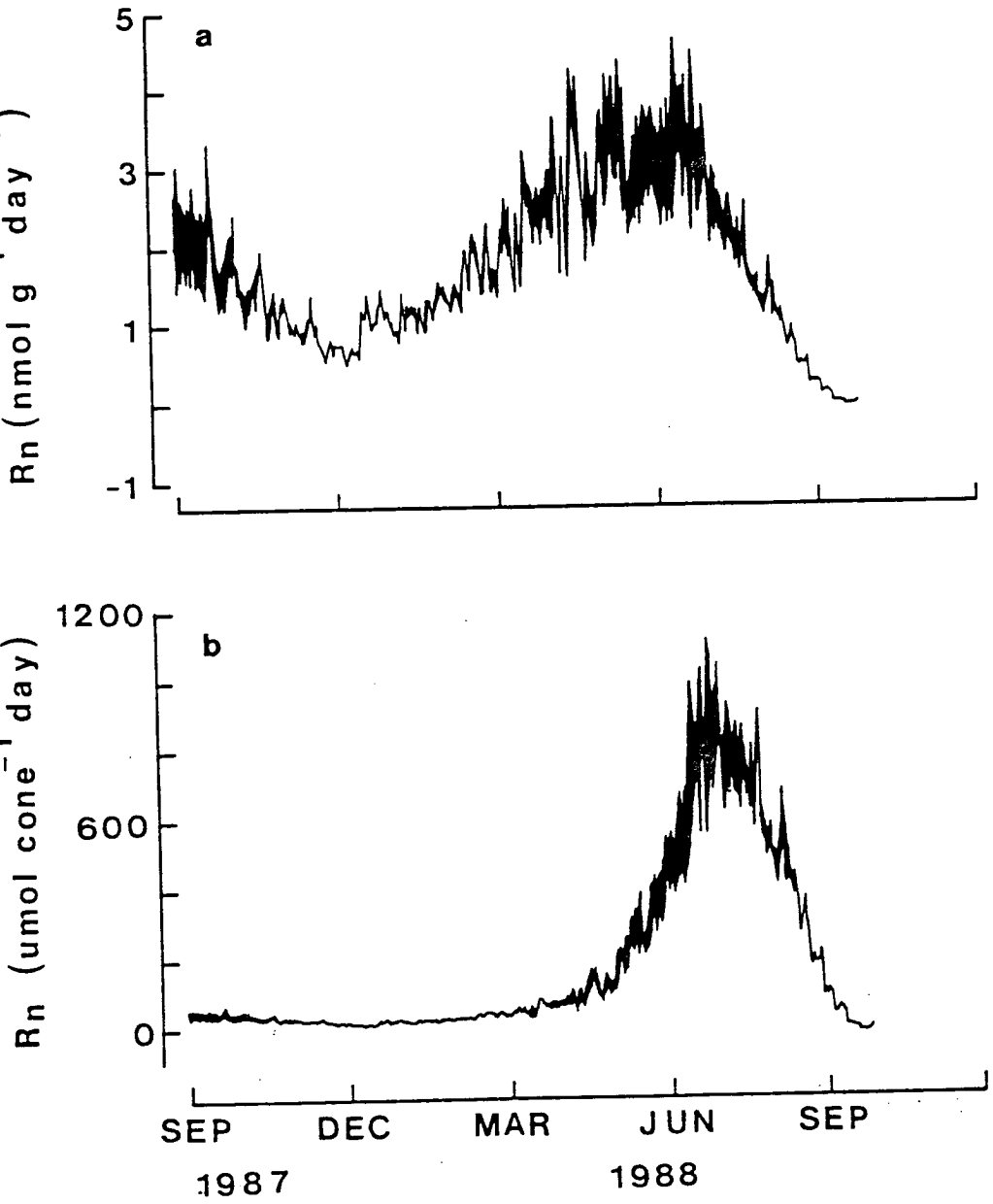


Figure 5.9 Estimated respiration rate of female cones of P. contorta from September 1987 to September 1988 a) per gram of tissue and b) per cone. Shaded area indicates the difference between dark (upper boundary) and net respiration (lower boundary).

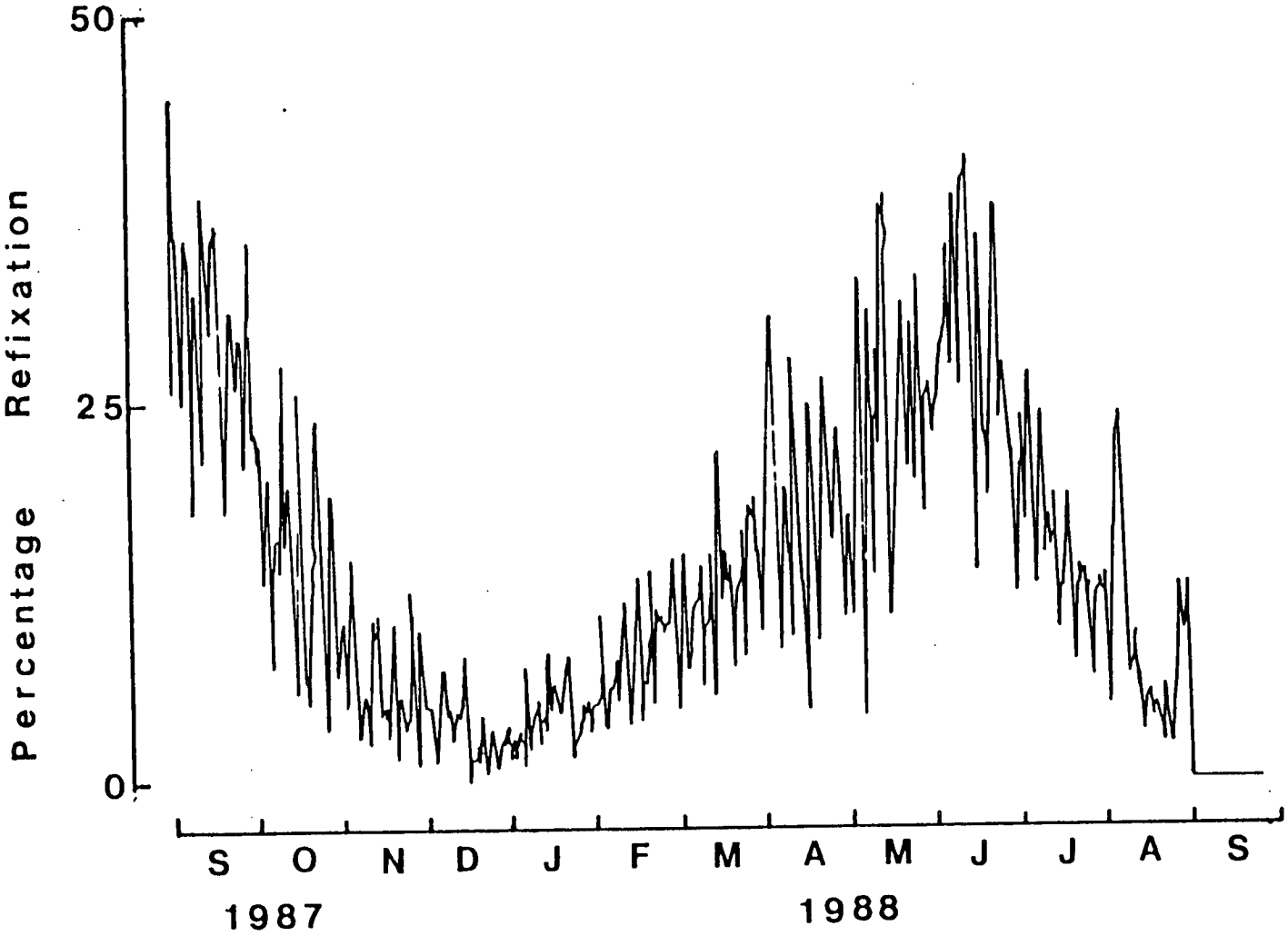


Figure 5.10 The percentage reduction in the dark respiration rate of female cones of P. contorta when illuminated.

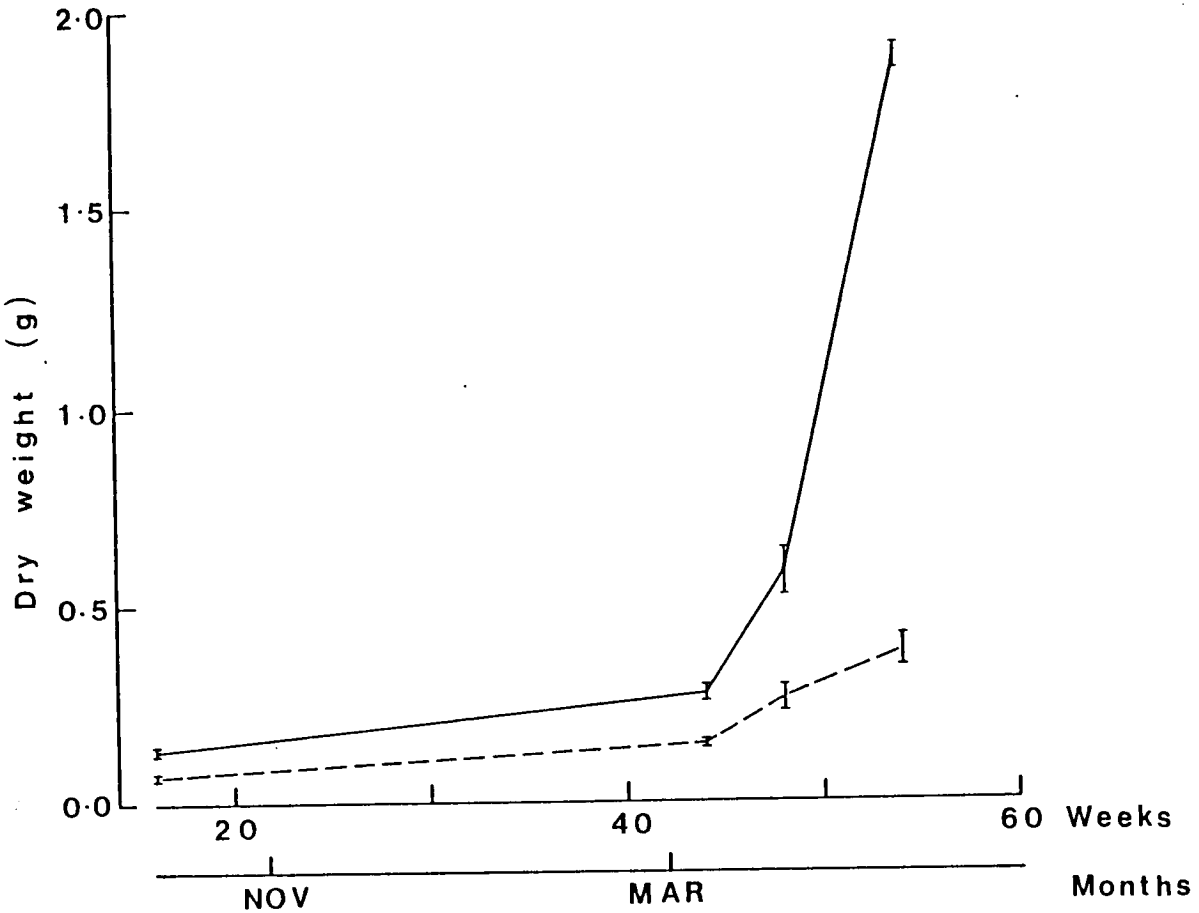


Figure 5.11 Dry weight of vegetative (----) and male cone-bearing (——)buds of P. contorta ($\pm$ one standard error of the mean).

Table 5.4 Percentage of the total dry weight enclosed in the assimilation chamber which was (a) male cones, (b) the remaining portion of the terminal bud, (c) lateral buds, and (d) the stem of: (i) male cone-bearing, and (ii) vegetative buds of P. contorta.

	WEEKS (from estimated date of male cone initiation - 1st June)			
	18	44	48	52
(i) Male cone-bearing buds				
male cone	66.7	65.0	69.1	80.2
terminal bud minus male cones	13.0	15.3	15.0	11.2
lateral buds	-	4.6	10.0	4.9
stem	20.4	15.6	5.8	3.7
(ii) Vegetative buds				
terminal bud	67.8	57.3	68.9	68.0
lateral buds	1.7	19.1	18.0	16.9
stem	32.2	23.6	13.0	15.1

Male cone-bearing buds had higher dark respiration rates in weeks 44 and 48 compared to vegetative buds. The highest dark respiration rate of male cone-bearing buds occurred at week 48, while vegetative buds had the highest dark respiration rate at week 52 (Table 5.5). Male cone-bearing buds had a lower refixation capacity compared with vegetative buds at week 52 (Table 5.5). Male cone-bearing buds had similar light response curves as vegetative buds at weeks 44 and 48. However, male cone-bearing buds showed little reduction in respiration rate when illuminated at week 52 (Figure 5.12).

Table 5.5 Mean dark respiration rate (nmol g^{-1} s^{-1}) and refixation percentage at quantum flux densities higher that 1500 μmol m^{-2} s^{-1} of male cone-bearing buds and vegetative buds of P. contorta at three sampling occasions in the early spring, weeks from the estimated date of cone initiation, i.e. 1st June (SE = one standard error of the mean).

	Male cone-bearing buds		Vegetative buds	
Week	respiration rate (SE)	% refixation	respiration rate (SE)	% refixation
44	-8.0 (0.34)	46	-5.1 (0.60)	42
48	-9.9 (0.53)	71	-5.1 (0.43)	73
52	-6.3 (0.31)	27	-6.1 (0.42)	84

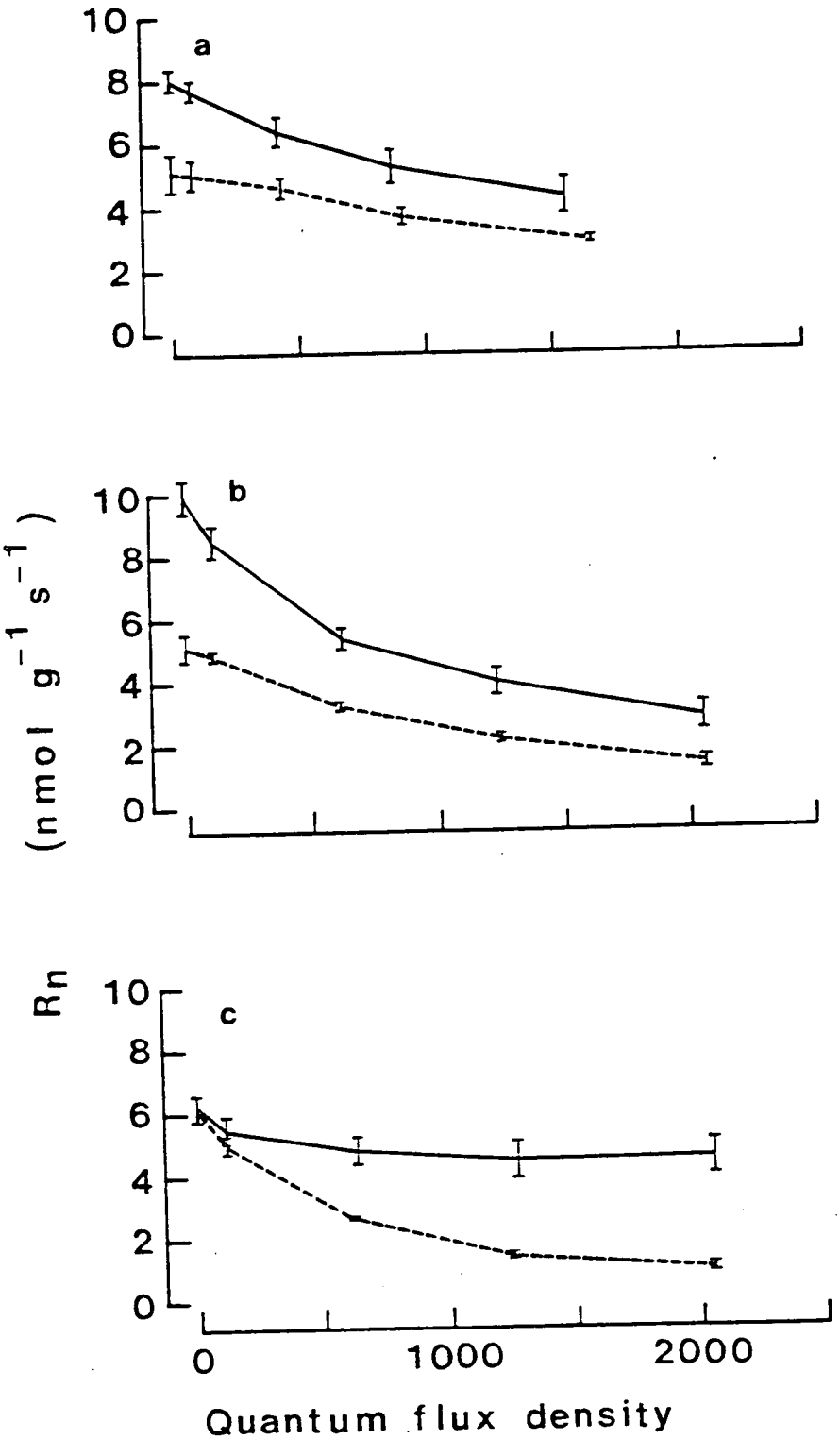


Figure 5.12 Respiration rate at five values of quantum flux density of vegetative (----) and male cone-bearing (——) buds of P. contorta in a) week 44, b) week 48 and c) week 52 from the estimated date of cone initiation (1st June).

5.6 DISCUSSION

5.6.1 FEMALE CONES

Although the n1 and n2 female cones were not sampled in the correct chronological sequence in this study, it is useful to consider the data in terms of the cone's stage of development. This was achieved by normalising the sample date to the 1st of June in the year of pollination. This approach allows the consideration of a logical sequence of female cone development.

Following pollination in late May-June female cones increased in surface area and dry weight very slowly during the autumn and winter. In the spring of the next year the cones expanded rapidly, more than doubling their dry weight between April and May (weeks 44 - 48 from the estimated date of pollination). The female cone continued to increase rapidly in both fresh and dry weight until mid-July, after which time the dry weight remained steady and the cone began to desiccate, prior to seed shed in late September. Similar increases in dry weight have been reported for other pines (Dickmann and Kozlowski 1969, Linder & Troeng 1981). The period of rapid cone growth in the spring and early summer, probably coincides with the recommencement of pollen tube growth in the spring. Female cones of P. contorta start to turn downwards in March and are fertilised around the end of May in southern Scotland (Alan Fletcher pers. comm.).

Respiration of plant material is commonly assumed to be the sum of two terms: (i) the basal metabolic or maintenance respiration, which is considered to be derived from the turnover of enzymes and membrane proteins, and (ii) synthesis or growth respiration, which is considered to be the respiratory cost of converting glucose to the various groups of compounds found in plants (Penning de Vries 1975, Evans 1975, McCree 1978, Lambers et al. 1983). Although there is no real distinction between these two terms in either biochemical pathways or products, the distinction is useful in explaining the three phases of respiration rates of female cones measured in the this study.

The dark respiration rate of female cones, measured in this study, can be considered as occurring in three phases:

(i) late autumn - early winter, which is a period of declining respiration rates. This probably coincides with the cessation of pollen tube growth (reduced growth respiration) and low winter temperatures, possibly reducing the rate of enzyme turnover and resulting in a low maintenance respiration cost (McCree 1978).

(ii) late winter - early summer, which is a period of high respiration rates. The increase in dark respiration rate between January and February (weeks 33 - 39) preceded the increase in measurable growth of the cone by a few weeks. This may well be the result of increased metabolic activity prior to cell expansion. Linder & Troeng (1981) have suggested that the period of maximum increase in respiration rate of P. sylvestris, which they found in July, corresponded with cone fertilisation. It seems likely that the cones of P. contorta are fertilised earlier in Scotland, as the dark respiration rate of the female cones were declining during July in this study. The maximum dark respiration rates of female cones was about 7 $nmol^{-1}\ g^{-1}\ s^{-1}$ (i.e. 1.1 mg $CO_2\ g^{-1}\ h^{-1}$) measured in May. This was about half the maximum respiration rate found by Linder & Troeng (1981) for Pinus sylvestris in June (2.1 mg $CO_2\ g^{-1}\ h^{-1}$).

(iii) late summer - autumn, which is a period of declining respiration rates. This correlates with the senescence of the cone which results in the reduction of both growth and maintenance respiration. Similar reductions in the respiration rates of fruits as they mature have been noted by several workers (e.g. Ching & Ching 1962, Opik 1980, Linder & Troeng 1981, Koppel et al. 1987).

Normally developed chloroplasts similar in structure to needle chloroplasts have been found in a narrow zone around the periphery of Pinus sylvestris seed scales (Kozubov 1985). They quote other work which show that the spectral characteristics of chlorophylls *a* and *b* from green cones and pine needles are analogous. In the present study, the refixation capacity of P. contorta female cones

(i.e. the reduction in respiration rate expressed as a percentage of the dark respiration rate) was close to 100% in the early stages of the cone development and declined slowly as the cone matured. This may be the result of reduction in chlorophyll content as the cones mature (Kozubov et al. 1985). Koppel et al. (1987) also found a steady decline in the refixation capacity from May to September for cones of Picea abies. However, Linder & Troeng (1981) found that the refixation capacity of P. sylvestris increased from around 30% to 75% during May and then was more or less constant until the end of September, when it suddenly declined. They suggested that the increase in refixation capacity in the early summer was similar to the increase in needle photosynthesis following the dormant winter period (see section 1.5.1). It is possible that the disturbance caused by low temperatures during the winter to the photosynthetic mechanisms of needles also operated on cones in Sweden but that the winter temperatures were not sufficiently sever in Scotland during this study.

In January, the respiration rate of all three replicates was slightly negative at quantum flux densities higher than 1500 μmol m^{-2} s^{-1}. This finding which, indicates the uptake of external CO_2, requires verification. Assuming the result is true, it implies that the cone was storing assimilates and possibly could have been exporting assimilates to the rest of the branch if it was still attached. Rook and Sweet (1971) found evidence when feeding $^{14}CO_2$ to cones of Pseudotsuga menziesii that a small amount of assimilates were possibly exported from the cone in spring. However, it should be remembered that such high quantum flux densities are seldom found in January in Scotland. Further works is required to determine the extent to which developing female cones export assimilates.

The model, devised in this study, estimates the net respiration rate of female cones throughout the second half of their life cycle and clearly shows the interactions between temperature, quantum flux density and the physiological state of the cone. Reed et al. (1976) have also suggested that the respiration rate of leaves increase exponentially with temperature and decrease exponentially with quantum flux density. They were uncertain if an asymptote

really existed for the reduction in respiration rate of leaves with increasing quantum flux density, female cones however clearly show such an asymptote. The model predicted that there would be very little difference between the dark and net respiration rates during the winter months because of the low temperatures and quantum flux densities even although female cones were most efficient at reducing the loss of respired CO_2 at this time. However during the spring and early summer, a significant difference was predicted between the dark and net respiration rates of the cones. Overall the average refixation capacity predicted by this model from September 1987 to September 1988 was 25%. This value is lower than the 31% estimated by Linder & Troeng (1981) which was probably the result of the high refixation capacities they found during the late summer.

It is interesting that Brown (1971) found almost twice the incidence of cone drop on the north side of the crowns of Scots pine grafts compared with the south side. As this difference occurred in both wind and controlled-pollinated cones it was not attributable to the pollen cloud. The refixation capacity of the cones may have contributed to this effect, if it is assumed that south-facing branches would receive higher quantum flux densities and consequently have an increased rate of CO_2 refixation.

The total respiratory losses of carbon, estimated by the model during the second half of the life cycle of a female cone weighing 4.5 g, was 0.71 g carbon. Other studies have estimated the reduction in stem wood by assuming direct and mutually exclusive competition for carbon between the stem wood and female cones (Linder & Troeng 1981, Koppel et al. 1987). Although cones are known to have a high competitive ability and to attract assimilates from other parts of the tree (Dickmann & Kozlowski 1968), it is not considered justified to assume that carbon utilised by the cone would otherwise have been available for stem growth. Male cones have been found to increase the photosynthetic rate of the needles immediately adjacent to them (Chapter 3 & 4) and, although this has not been demonstrated for branches bearing female cones, it has been suggested that female cones may elicit an increased photosynthetic rate on the surrounding branches in a similar manner (Chapter 3).

5.6.2 MALE CONES

Compared with vegetative buds, male cone-bearing buds had a larger surface area and dry weight 18 weeks after the estimated date of initiation. By week 52 (late May of the following year) male cone-bearing buds had 80% more dry weight than vegetative buds. It was not possible to separate the male cones and measure their respiration rate independently from the surrounding bud and stem tissue. However the male cone portion of the bud always represented more than 65% of the dry weight of the material in the chamber. While it may be of academic interest to separate the respiration rate of the male cone from that of the vegetative portion of the same bud, as male cones do not occur in isolation in nature, it is considered valid in this study to consider the whole bud complex. Future experiments should, however, estimate the refixation of the stem separately from the bud. This would allow the respiration rate of the male cone-bearing bud alone to be estimated. This was not attempted in this study because time in the photosynthesis laboratory was severely limited.

The dark respiration rate of both male cone-bearing and vegetative buds increased in the spring. This increase is probably the result of increases in both the growth and maintenance components of respiration (Kozlowski & Gentile 1958). Male cone-bearing buds had higher dark respiration rates during the early spring compared with vegetative buds and this probably reflects their higher growth rate. There was, however, no difference in the respiration rate of the male cone-bearing and vegetative buds just prior to pollen shedding, presumably because the cones were approaching maturity. This is perhaps similar to the reduction in the respiration rates of the female cones at senescence.

Male cone-bearing buds and vegetative buds had a similar refixation capacity until just before the male cones shed their pollen. This suggested that the male cones were as efficient at refixing CO_2 as young needle primordia. To estimate the carbon cost of male cones throughout their life cycle, it would be necessary to measure the

respiration rate of male cone-bearing buds from initiation to abscission. This preliminary study has shown the feasibility of such an exercise but unfortunately it was not possible within the scope of this project. The respiration rates of the vegetative and male cone-bearing buds appear to respond to changes in light in much the same way as female cones, therefore, it is probable that the model derived in this chapter for female cones could be used to estimate the total carbon required for male cones. However, independent estimates of the parameters would be required. It is also possible that the equations utilised in this study could be used to estimate the net respiration rate of tree bark which has been found to have reduced rates of respiration in light (e.g. Linder & Troeng 1980, Benecke 1985).

5.6.3 CONCLUSION

Female cones and male cone-bearing buds have a similar range of respiration rates per unit of dry weight. However as the female cones are heavier and remain on the tree for a longer period they may be a larger drain on the carbon reserves of the tree.

In conclusion, this study shows that both female cones and male cone-bearing buds have significantly reduced respiration rates when illuminated and consequently contribute to their own carbon economy. Furthermore, as both have stopped growing before the period of peak cambial growth (Deans & Ford 1986), it is possible that the carbon requirements of neither male or female cones significantly reduce tree stem wood increment. It is not possible, from this study, to estimated the influence of reproductive structures on the growth and yield of whole plants. In order to construct a complete carbon budget for whole plants a system was designed which is explained in Chapter 6.

5.7 SUMMARY

1) Female cones increase in surface area and weight very slowly during the autumn and winter following pollination. This period is followed by a phase of rapid growth until early August. The cones

then begin to dry out and shed their seeds in late September and early October.

2) During the late autumn and early winter the dark respiration rate of female cones decrease gradually from 4 nmol g^{-1} s^{-1} to 2.5 nmol g^{-1} s^{-1}. The dark respiration rate then almost doubles within 4-6 weeks and remains high until the female cone begins to dry out during the late summer. The dark respiration rate of the female cones declines toward the autumn as the cone begins to senescence.

3) The pattern of cone growth and the rates of dark respiration throughout the life cycle of the female cone are consistent with the current theories of growth and maintenance respiration. The dark respiration rate declines through the winter as the temperature falls, thus resulting in lower respiration rates and presumably reduced enzyme and protein turnover. The phase of maximum dark respiration rate precedes the period of maximum growth rate of the female cone by a few weeks. This is probably the result of a period of high metabolic activity before the cone measurably swells. The phase of declining dark respiration rate closely follows the drying out of the cone prior to seed shedding.

4) The respiration rates of female cones decreases exponentially with increasing quantum flux density. The ability of the cones to reduce their respiration rate when illuminated (refixation capacity) decreases as they mature. This reduction is from around 100% at 14 weeks after the estimated date of pollination (1st June) to zero by week 69 when the seeds are dropped.

5) Although the female cones are most efficient at refixing respired CO_2 in the winter, the model predicting the net respiration rate of female cones, shows that as a result of low quantum flux densities in the winter there is little difference between the dark and net respiration rate of female cones. However, in the spring and summer female cones have a daily refixation capacity of 27%.

6) Male cone-bearing buds initiated in mid-summer, increased in surface area and dry weight slowly during the winter. They then

rapidly increase in weight and surface area the following spring, prior to shedding their pollen.

7) Male cone-bearing buds have a higher dark respiration rate in the spring following initiation, but the dark respiration rate of male cone-bearing and vegetative buds is similar 2-3 weeks prior to pollen shed.

8) Both male cone-bearing and vegetative buds have a reduced respiration rate when illuminated. The refixation capacity of both is similar until just before the male cones shed their pollen.

9) The reduced refixation capacity of male cone-bearing buds just before they shed their pollen, is similar to female cones just before they shed their seed.

10) It is concluded that female cone and male cone-bearing buds have significantly reduced respiration rates when illuminated and consequently contribute to their own carbon economy.

CHAPTER 6

DEVELOPING A POTTED PLANT SYSTEM WITH AND WITHOUT REPRODUCTIVE STRUCTURES

6.1 AIM

To construct a complete carbon budget for P. contorta plants, it is necessary to be able to quantify the carbon inputs and outputs of all the components of the plant (e.g. needles, branches, stem and roots). The aim of this study was to develop a potted plant system which would allow these components to be measured.

6.2 INTRODUCTION

The previous chapters have shown that reproductive branches have: (i) more total dry weight then vegetative branches (Chapter 2), (ii) that, in the autumn, needles on the parent shoots of male cone-bearing branches have a higher photosynthetic rate than equivalent vegetative branches (Chapter 3 & 4) and (iii) that male and female cones have significantly reduced net respiration rates when illuminated (Chapter 5). The influence of these factors on the total carbon budget of the trees could not be determined in the above studies, because the CO_2 exchange and the accumulation of dry matter was not recorded simulatenously in the needles, branches, stem, roots and cones of the trees. It was the aim of this study to develop a system which would allow all the components of a tree to be measured and the dynamic interactions between the parts to be monitored. The components of the system are outlined in Figure 6.1.

Two main approaches were tried in this study: (i) to take rooted cuttings and induce them to initiate cones, and (ii) to take cuttings with cones already initiated and induce them to root. These two approaches will be considered separately within this chapter:

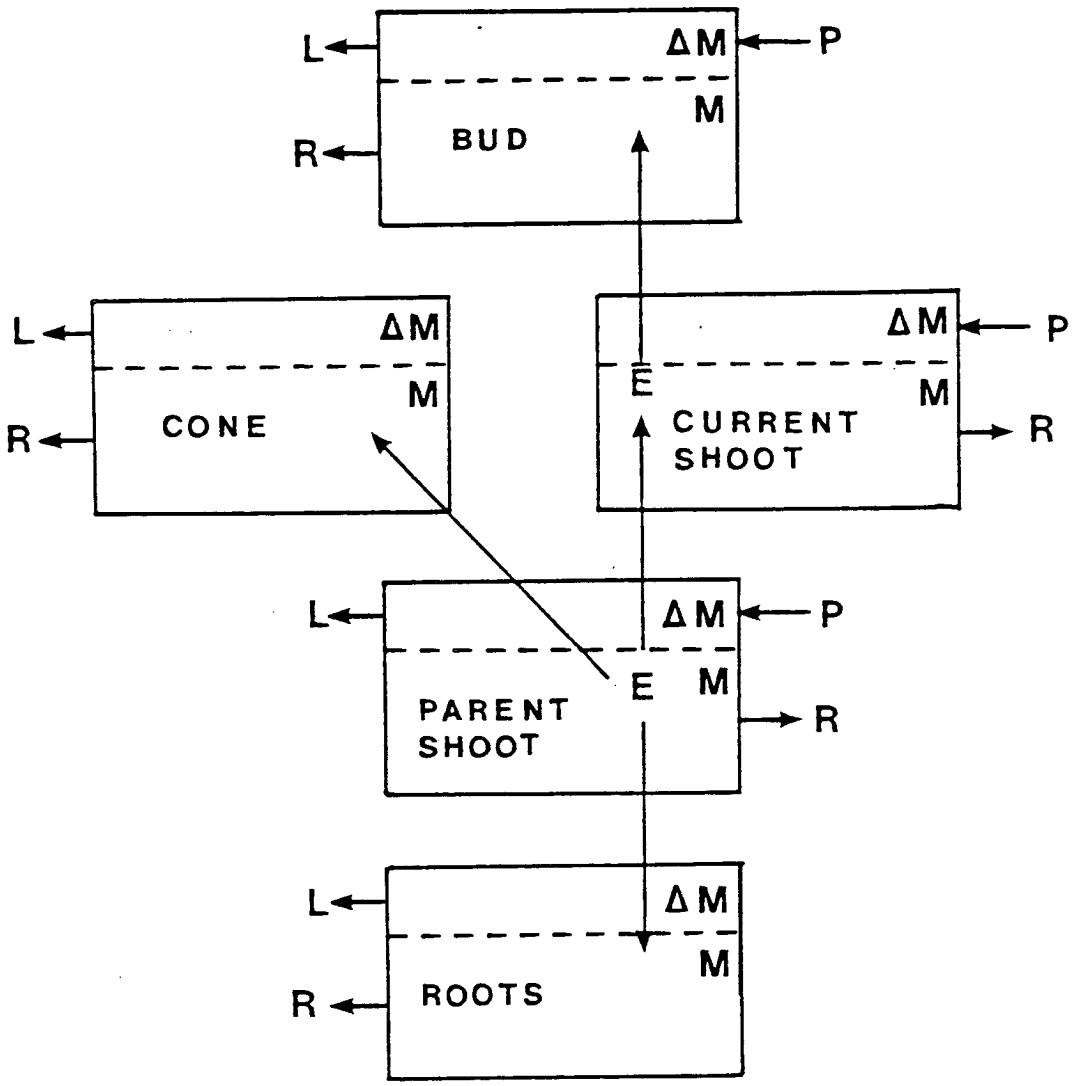


Figure 6.1 Diagram of the parts of a reproductive cutting showing inputs and outputs of carbon, E=export, L=litter, M=existing carbon, Δ M=change in carbon, P=photosynthesis, R=respiration.

(i) Cone induction; the influence of daylength and plant growth regulators on the initiation of male and female cones was investigated to determine if they would be suitable treatments to induce cones on potted plants without altering the basic morphology of the plants (section 6.3).

(ii) Rooting cuttings; because cuttings of P. contorta had been routinely propagated at I.T.E. since 1975, it was decided to investigate this method first, followed if neccessary, by grafting as demonstrated by Rook & Sweet (1971) for female cones of Pseudotsuga menziesii. Cuttings were taken at three stages in the development of the cones during 1984, to determine if it was possible to root cuttings and retain viable cones (section 6.4). In addition five potting media were investigated to determine the most suitable method of managing the reproductive cuttings (section 6.5).

Following the inital success of the reproductive cuttings in 1984 similar branches growing in the field were bark-ringed to determine if the previous year's growth was sufficient to support normal cone development. This was done because the cutting system required that the cones develop with only the assimilates produced in the parent branch (i.e. no importation from the rest of the tree) (section 6.6). Following the encouraging results obtained in 1984 vegetative and reproductive cuttings were again taken in 1985 (section 6.7) and 1986 (section 6.8).

6.3 CONE INDUCTION ON ROOTED CUTTINGS

There are many treatments used by tree breeders and plant physiologist, to induce conifers to produce cones (see reviews by Purtich 1972, Lee 1979, Longman 1985, Ross & Pharis 1985, Owens & Blake 1985). Short days have been associated with the initiation of female cones (Giertych 1967, Longman 1982), while long days have been associated with the initiation of male cones (Giertych 1967). Female and male cones are found in fairly distinct zones within the crowns of conifers (Wareing 1958, Dick et al. 1985, Dick et al. in prep.). Generally the female cones are located at the distal region of branches, while male cones are located at the proximal region of

branches. In addition, female cones are generally found higher in the tree crown than male cones (Ross et al. 1983, Longman & Dick 1985). These gradients of sexuality have been associated with both the vigour of the cone-bearing branch and the level of plant growth regulators within the tree crown. Auxins have been associated with the presence of female cones while members of the gibberellin family have been associated with male cones (Kopcewicz et al. 1977, Galoch & Michniewicz 1978, Moritz et al. 1989). The hypothesis that short days and auxins induced female cones while long days and gibberellins induce male cones was tested in this study.

6.3.1 MATERIALS

A total of 96 cuttings were selected in the spring of 1984 from material rooted in 1982. The basal needles had been removed and a clean slanting cut made at the base of the cutting, which were then dipped in the commerical rooting powder ('Strike', May & Baker Ltd. Dagenham, England) and placed under a mist propagation system described in section 6.4.2. Forty-eight plants each of two clones were chosen for this study: (i) clone 8021, the stockplants of which produced regular crops of both female and male cones, and (ii) clone 8983 which was a predominately male cone-bearing genotype.

The stockplants of clone 8021 were growing in the nursery plots of the Forestry Commission's Northern Research Station, Bush Estate, near Edinburgh. This clone originated from cuttings rooted in 1971 from a seedling growing at Broxa Forest, England. It was an inter provenance hybrid (Queen Charlotte Island x Olympics). The stockplants of clone 8983 were growing at the 'Farfield' plot of I.T.E., Bush Estate, near Edinburgh. They were cuttings collected and rooted in 1972 from a tree of Queen Charlotte Island provenance, growing at Selm Muir Forest, Scotland.

The plants chosen for this study were approximately 0.3-0.6 m in height and were potted in 1.5 litre square pots. Within each clone, the plants were selected into groups of six depending on the number of growing apices on each plant. Each plant, within a group of six, was then randomly assigned to one of the six treatments.

6.3.2 METHODS

The plants were treated in a 2 x 3 factorial experiment with 16 replicates: 2 daylength treatments (short and long) X 3 injection treatments (auxin, gibberellin and control (industrial methylated spirts)).

Two walk-in growth rooms at the Department of Forestry and Natural Resources of Edinburgh University, were used to provide growing condition with different daylengths. The main lights of both growth rooms were on for eight hours daily and provided an average of 350 μmol m^{-2} s^{-1} (red to far red ratio 2.0). Illumination was provided by 20 discharge lamps (MBI/H 400 W, Thorn Lighting LTD. Glasgow) and 16 tungsten light bulbs. The daylength was extended in one of the rooms by the tungsten bulbs (15 μmol m^{-2} s^{-1} red to far red ratio 1.3) to give a total daylength of 19.5 hours. The temperature was controlled in each room at 18 °C for eight hours (corresponding to the period of main light illumination) and 13 °C during the remaining 16 hours. The humidity in each room was controlled between 65% and 80%.

The plant growth regulators, injected into the base of the stem, were: (i) a mixture of approximately 57% GA_4 and 43% GA_7 (GA) supplied as an experimental sample by the Plant Protection Division of I.C.I. (Jealott's Hill Research Station, Backnell Berks, England) and (ii) α-naphthylene acetic acid (NAA) supplied by Sigma Chemical Company (Fancy Road, Poole, Dorset, England). Both these chemicals were dissolved in IMS (industrial methylated spirit GPR, about 90% w/w total alcohol, BDH, Thornliebank, Glasgow, Scotland). Each plant was injected with 0.5 μl of solution into each of two holes by the Simple Hole Method described by Dick and Longman (1985). The solutions were: (i) IMS alone (IMS), (ii) 400 μg GA dissolved in 10 μl IMS and (iii) 100 μg NAA dissolved in 10 μl IMS.

The plants were injected on 11/6/84 and transfered to the growth rooms at the University on 12/6/84. They remained in the growth rooms for 85 days and were transferred on 6/9/84 to a frost-free glasshouse at I.T.E., Bush Estate, near Edinburgh.

The height of the 1984 terminal shoot was measured when the plants were removed from the growth room. In addition the length of 15 needles per terminal shoots were measured. The height of the terminal bud was also measured when the plants were removed from the growth room on the 6/9/84 and again on 10/7/85 when shoot extension has ceased. The number of cones and cone-bearing buds was assessed on 16/4/85.

6.3.3 RESULTS

Generally, only the plants injected with GA produced cones. The exception was one control plant grown in the long day growth room which produced one female cone compared with a total of 33 female cones on 11 GA treated plants (Table 6.1). Sixty-two percent of the plants injected with GA and grown in the short day growth room produced female cones while 19% produced male cones. In contrast 19% of the plants grown in the long day growth room produced female cones and 31% produced male cones. However the total number of buds which grew in 1985 was slightly reduced in the GA treated plants (Table 6.1).

There was little difference between treatments in the mean terminal shoot elongation in 1984 (Figure 6.2). However, the needles elongating under long days were approximately three times longer than those in the short day treatment (Figure 6.3). In early September 1984, when the plants were removed from the growth rooms, the terminal buds which were initiated in long days were approximately 30% longer than the plants grown under short days (Figure 6.4). The GA treated plants had the longest terminal buds in September 1984, regardless of daylength treatment.

In 1985, the plants which had been subjected to long days had significantly longer terminal shoots compared with equivalent treated plants grown under shortdays (Figure 6.4) and a significantly greater number of needles per terminal shoot (Figure 6.5). The GA treated plants were significantly taller and had a more needles than the IMS treated plants regardless of day length treatment. While the NAA treated plants had shorter terminal shoots

than the IMS treated plants and had slightly fewer needles on the terminal shoot.

Table 6.1 The (i) number of P. contorta plants which coned (out of 16), (ii) total number of cones, (iii) total number of cone-bearing buds produced and (iv) the mean number and standard error of growing buds on plants grown in 8 h daylength (SD), or 19.5 h daylength (LD) and injected with either industrial methylated spirit (IMS), α-naphthylene acetic acid (NAA) or a mixture of gibberellin 4 and 7 (GA).

(i) No. plants coning

Daylength	IMS		NAA		GA		Total	
	F	M	F	M	F	M	F	M
SD	0	0	0	0	10	3	10	3
LD	1	0	0	0	1	5	2	5
Total	1	0	0	0	11	8	12	8

(ii) No. cones per treatment

Daylength	IMS		NAA		GA		Total	
	F	M	F	M	F	M	F	M
SD	0	0	0	0	32	8	32	8
LD	1	0	0	0	1	219	2	219
Total	1	0	0	0	33	227	34	227

(iii) No. of coning buds per treatment

Daylength	IMS		NAA		GA		Total	
	F	M	F	M	F	M	F	M
SD	0	0	0	0	12	3	12	3
LD	1	0	0	0	1	17	2	17
Total	1	0	0	0	13	20	14	19

(iv) Mean number of growing buds per plant (standard error)

Daylength	IMS	NAA	GA	Total
SD	11.6 (1.2)	9.0 (1.0)	6.9 (0.7)	9.2 (0.6)
LD	9.3 (0.6)	9.7 (0.5)	8.9 (0.6)	9.3 (0.3)
Total	10.4 (0.7)	9.4 (0.6)	7.9 (0.5)	9.2 (0.3)

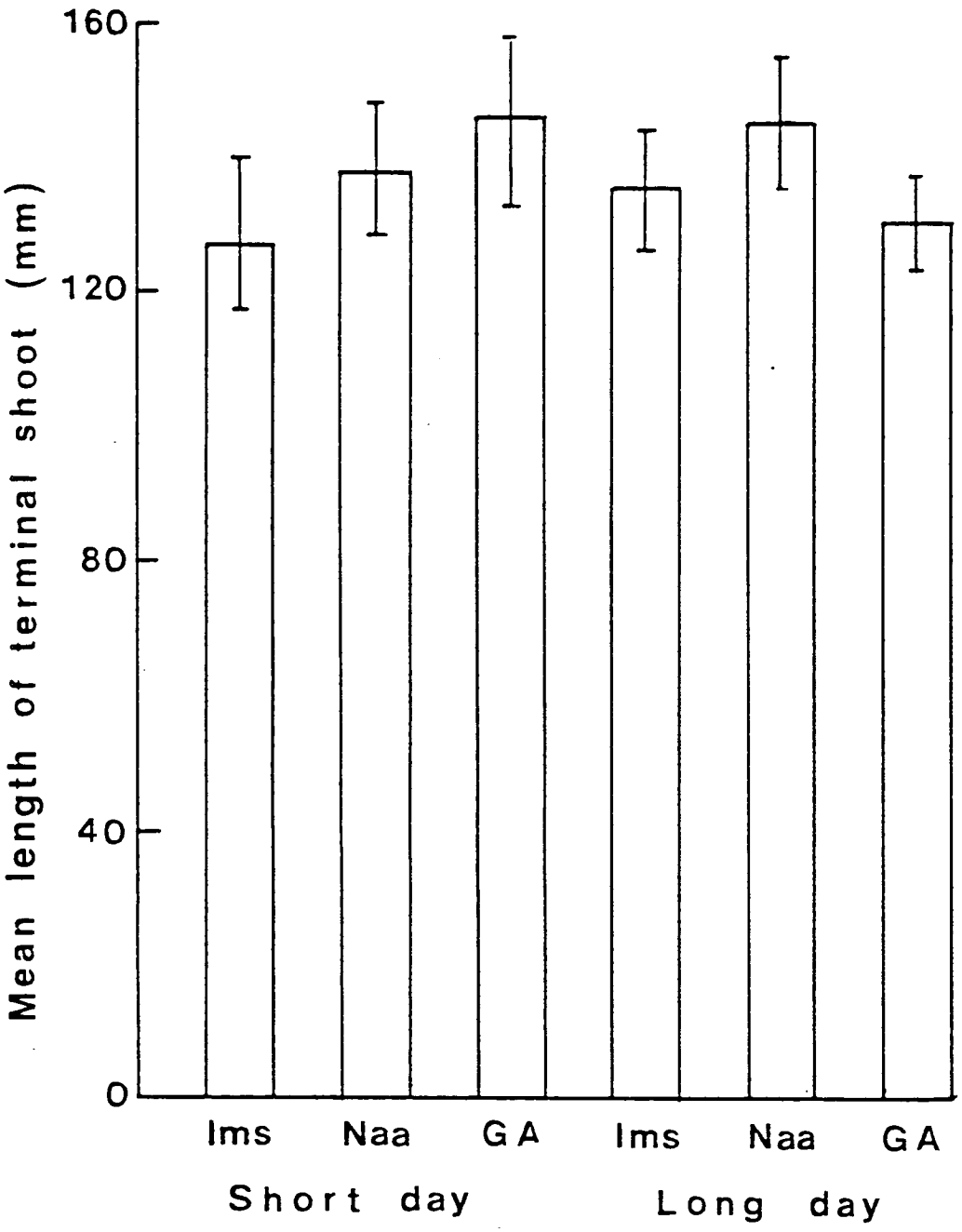


Figure 6.2 Mean height of the terminal shoot of P. contorta plants grown in short (8 h) or long (19.5 h) daylengths and injected with either industrial methylated spirit (Ims), ∝-naphthylene acetic acid (Naa) or a mixture of gibberellins 4 and 7 (GA).

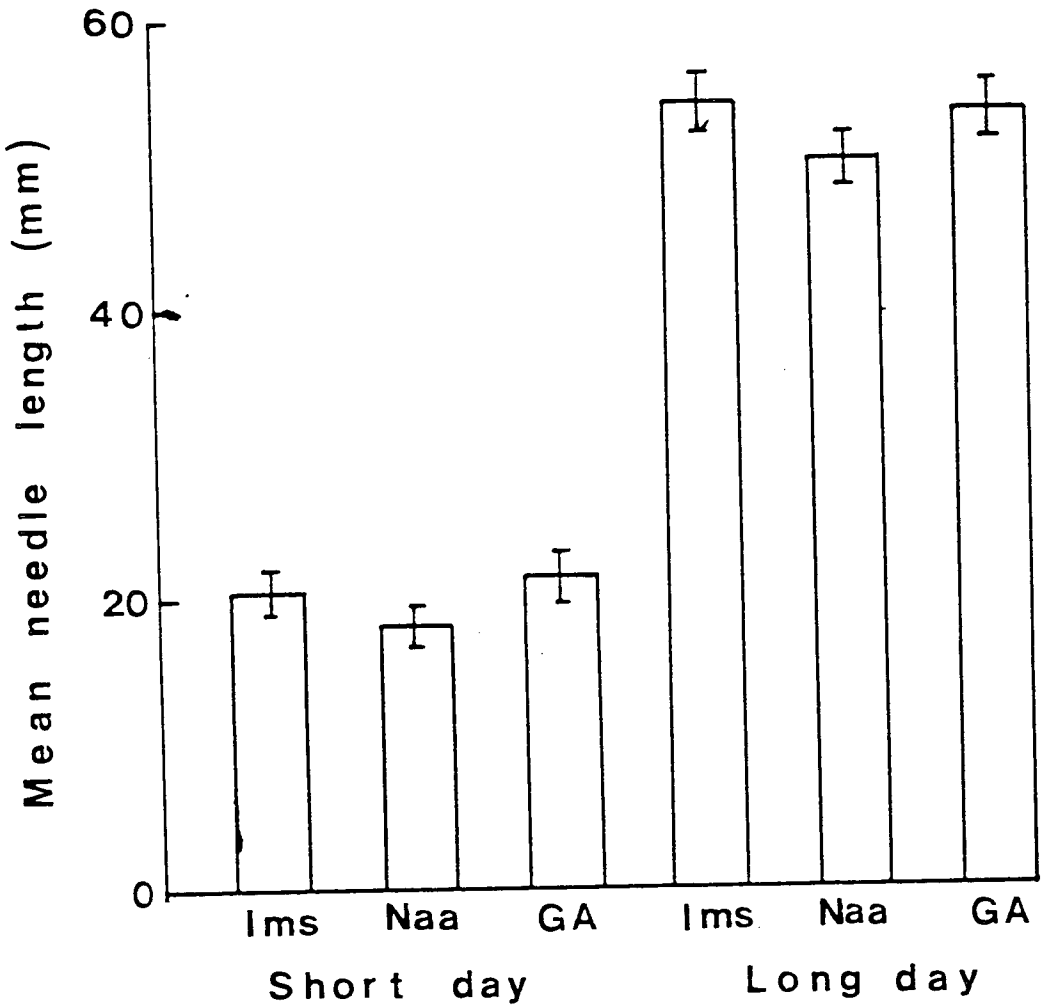


Figure 6.3 Mean length of needles on the terminal shoot of P. contorta plants grown in short (8 h) or long (19.5 h) daylengths and injected with either industrial methylated spirit (Ims), ∝-naphthylene acetic acid (Naa) or a mixture of gibberellins 4 and 7 (GA).

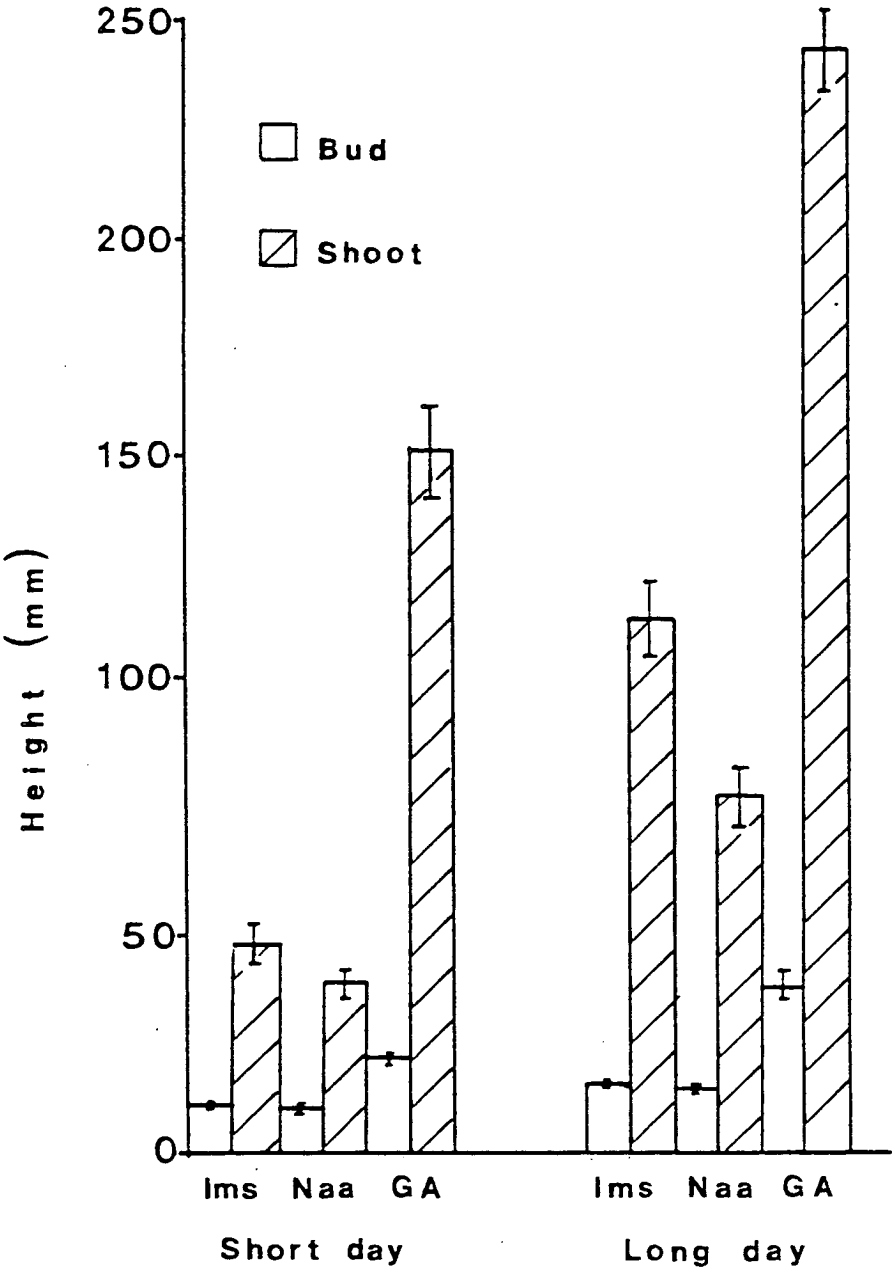


Figure 6.4 Mean length of the terminal bud and the subsequent terminal shoot of P. contorta plants grown in short (8 h) or long (19.5 h) daylengths and injected with either industrial methylated spirit (Ims), α-naphthylene acetic acid (Naa) or a mixture of gibberellins 4 and 7 (GA).

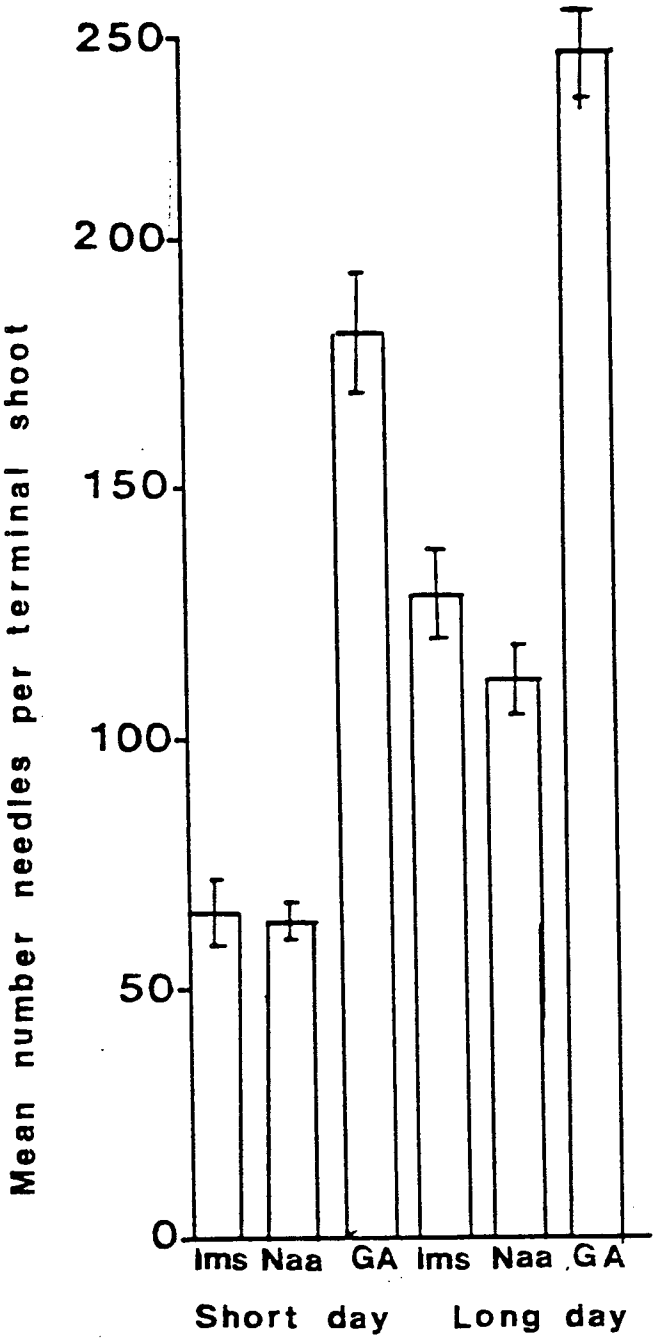


Figure 6.5 Mean number of needle primordia initiated on the terminal shoot of P. contorta plants grown in short (8 h) or long (19.5 h) daylengths and injected with either industrial methylated spirit (Ims) α-naphthylene acetic acid (Naa) or a mixture of gibberellins 4 and 7 (GA).

6.3.4. DISCUSSION

The results of this study generally support one part of the original hypothesis, namely that plants grown in short days produced more female cones while plants grown in long days produce more male cones (see section 6.3.1). However the number of cones produced in this study was small. To some extent this probably reflects the small size of the plants which had only 7-12 growing buds. However even on reproductive plants only 10% - 30% of the buds formed cones. The plant growth regulator NAA did not influence the initiation of reproductive buds, while GA appeared to increase the frequency of both male and female cones. The sex promoted appeared to be determined by the daylength in which the plants were growing.

In comparison with long days, plants grown under short days had a 30% reduction in the mean needle length. As the quantum flux density of the period of extended daylength was below the light compensation point found in Chapter 3 and 4. It is unlikely that the increase in vegetative growth was the result of an increase in the availability of assimilates.

The increase in shoot extension found on the plants grown in the extended daylength in the year following treatment probably reflects the increase in the number of needle primordia; while the difference in needle length was probably the result of increased cell expansion. Similar results have been observed by other workers (see Longman 1982) and have been associated with the extended daylength and the light quality (see reviews in Pirson & Zimmermann 1983).

The aim of this study was to produce cones on small potted plants to determine the influence of male and female cones on the carbon balance of P. contorta plants. Although cones were induced on small potted plants the effects of the these treatments on the vegetative growth of the plants (i.e. needle number and elongation) were undesirable because they altered the allocation of assimilated within the plants. Therefore, the morphological effects associate with the daylength and GA injection make these treatments unsuitable as techniques for inducing cones on rooted cutting of P. contorta in

order to study the influence of reproductive structures on the carbon balance.

6.3.5 SUMMARY

1) Plants grown from June to September in a growth room with a daylength of 8 hour (short day treatment) had significantly shorter needles than plants grown in a 19.5 hour daylength (long day treatment).

2) Plants subjected to long days during the period of needle primordia initiation produced more needle primordia on the terminal shoot and subsequently a longer terminal shoot than plants grown under short days.

3) This study supports the hypothesis that female cones are promoted by short days, while male cones are promoted by long days. GA generally enhance the level of coning in plants and the sex of cone produced depended on the daylength the bud was subjected to NAA promoted neither female or male cones.

4) The treatments used in this study, while giving encouraging results in terms of cone initiation, unfortunately resulted in distinct morphological changes in shoot length, needle number and needle length. It was therefore concluded that these treatments would not provide suitable material for studies on the carbon budget of P. contorta plants.

6.4 VEGETATIVE PROPAGATION OF REPRODUCTIVE CUTTINGS

A cutting with one and two year old needles was the simplest system envisaged which would resemble a 'tree'. There is a great deal of evidence (see section 1.5). that needles on the parent shoot supply the roots with assimilates early in the growing season before suppling assimilates for the current year's shoot extension. Once the current year's shoots are 33-50% expanded they and the parent shoots export assimilates to the rest of the tree. In addition, the one and two year needles are the most productive on the tree (Jarvis

& Sandford 1986). Therefore a cutting with both parent and current year's growth is a defined system that would be expected to give a reasonable approximation of a tree. This system should allow the inputs, outputs and the storage of carbon by the plant to be measured and a complete carbon budget to be determine for P. contorta, (i.e. it should be possible to measure all the components shown in Figure 6.1).

The reduction in rooting potential with the age of the stockplant has been noted by many workers (see reviews Struve 1983, Bogdanov 1984) and has been correlated in conifers with the onset of cone-bearing (Steele 1985). However, cuttings of P. contorta from cone-bearing stockplants have been routinely propagated at I.T.E. since 1975. The method is essentially mist propagation of lignified winter cuttings taken in the early spring (A Longman pers. comm.). Female cones were removed prior to setting the cuttings, while shoot with male cone-bearing buds were avoided. Cones were considered likely to reduce the cutting's ability to form roots, by providing an alternative sink for assimilates.

6.4.1 MATERIALS

Cuttings were taken at three stages in the development of the cones during 1984: (i) in the spring which was the 'normal' time for production cuttings of P. contorta at I.T.E. and is the time of year when female and male cone-bearing buds could be easily identified, (ii) in mid summer when the n1 female cone had been pollinated, and (iii) in the autumn when the n1 female cone had been pollinated and the male cone-bearing buds could be identified.

A standard system of nomenclature was adopted throughout this chapter. Cones and vegetative structures were coded as follows F = female, M = male and V = vegetative. As the cuttings were taken at three different times of year the characteristics of the cuttings varied. In the spring and autumn the cuttings consisted of a bud and a shoot, while in the summer the cuttings were composed of both the current and previous year's shoot. The coding gives first the condition of the current year's shoot or bud and then the previous

year's shoot (e.g. VV = vegetative bud or current year's shoot on a vegetative parent shoot, MV = a male cone-bearing bud on a vegetative parent shoot). Unfortunately with the autumn cuttings it was often not possible to identify the sex of the overwintering bud (U = unidentified bud). The source of reproductive material varied depending on the availability of suitable cuttings. Material from three tree's (8020, 8971 and 8972) was collected in 1984 (Table 6.2).

6.4.2 METHODS

Cuttings were detached from the tree with secateurs and immediately dipped in a fungicide (Mildothane, Dow Agriculture, Hitchin, U.K.). They were put into moist polythene bags and stored in a cold room (4 °C) until they could be processed the same day. The needles from the bottom 2-3 cm of stem were removed with scissors prior to recutting the base of the cutting at an oblique angle with a scalpel Cutting were then dipped into a commercial rooting powder (Strike, May & Baker Ltd. Dagenham, England). The cuttings were placed at 8 cm spacing into a mixture of 75% 3-5 mm grit and 25% medium sphagnum peat. The mist frequency and duration were controlled on a timer mechanism (I.T.E., Engineering Services, Bush Estate, Penicuik, Scotland) operated manually by the glasshouse staff at I.T.E. depending on local weather condictions. The number of cuttings rooted was recorded 3 - 5 months later.

In early May 1984 the male cones shed their pollen, and the female cones emerged while the cuttings were still in the mist bench. The newly emerged female cones were manually pollinated every day between 6/5/84 and 31/5/84. Fresh pollen was collected every 2-3 days from potted plants growing in a frost free glasshouse as the trees out of doors did not shed pollen until early June that year.

Table 6.2 Provenance and planting site of the P. contorta trees used as a source of cutting material between 1984 and 1986.

Tree number	Provenance	* Site of planting
8001	Ballyknoe, Ireland	FC Nursery
8004	Unkown	FC Nursery
8020	Queen Charlotte Island X Olympics hybrid	FC Nursery
8021	Queen Charlotte Island X Olympics hybrid	FC Nursery
8964	Takysie, B.C.	ITE Nursery
8965	Annette Island, Alaska	ITE Nursery
8966	Smithers Bulkey River, B.C.	ITE Nursery
8967	Cedarvale Skenna River B.C.	ITE Nursery
8968	Takysie, B.C.	ITE Nursery
8969	Cedarvale Skenna River B.C.	ITE Nursery
8971	Cedarvale Skenna River B.C.	ITE Nursery
8972	Cedarvale Skenna River B.C.	ITE Nursery
8974	Skagway, Alaska, clone 12	ITE Farfield
8975	Skagway, Alaska, clone 4	ITE Farfield
8983	Queen Charlot Island, Canada.	ITE Farfield
8985	Takysie, B.C.	ITE Nursery
8994	Cedarvale Skenna River B.C.	ITE Nursery

* ITE Nursery - the nursery plot at the Institute of Terrestrial Ecology, Bush Estate, near Edinburgh, Scotland, The plot was planted as a provenance trial in 1972 with seedlings sown in 1971.

ITE Farfield plot - planted in 1973 with cuttings vegetatively propagated in 1972 from material collected from Shin near Lairg, Scotland.

FC Nursery - the nursery plot of the Northern Research Station of the Forestry Commission, Roslin, Edinburgh, Scotland. The plot was planted in 1972 with cuttings vegetatively propagated in 1971.

6.4.3 RESULTS AND DISCUSSION

Although reproductive cuttings rooted when collected in April (Table 6.3), and appeared to have been successfully pollinated (60-70%), all of the female cones later aborted. This may be because the mist interfered with the pollination mechanism, for example by diluting the pollination droplet (see section 1.8.2). The male cones shed their pollen before the cuttings had rooted. Cuttings taken in the early spring were therefore, not suitable as material to construct a carbon budget for P. contorta plants.

Table 6.3 Number of vegetative and reproductive cuttings and the number and percentage of cuttings rooted from P. contorta trees at three stages in the development of the cones. Material was collected: (i) spring - 16 to 17/4/84 and potted 10/9/84, (ii) summer - 26/6/84 and potted 20/11/84 and (iii) autumn 1/10/84 and potted 12/2/84. Type of cutting was coded as follows; V=vegetative, F=female cone, M=male cone and U=unidentified bud, the first letter describes the bud followed by the type of parent shoot.

Clone	UF	UV	FV	MV	MM	MF	VV	TOTAL
(i) Spring								
8020								
No. cuttings	30	50	192	50	50	0	0	372
No. rooted	6	27	48	15	12	0	0	108
% rooted	20.0	54.0	25.0	30.0	24.0	0	0	29.0
(ii) Summer								
8020								
No. cuttings	0	0	27	0	22	0	0	49
No. rooted	0	0	0	0	0	0	0	0
% rooted	0	0	0	0	0	0	0	0
8972								
No. cuttings	0	0	124	0	0	0	121	245
No. rooted	0	0	4	0	0	0	24	28
% rooted	0	0	3.2	0	0	0	19.8	11.4
(iii) Autumn								
8971								
No. cuttings	28	26	0	26	0	0	0	80
No. rooted	0	0	0	0	0	0	0	0
% rooted	0	0	0	0	0	0	0	0
8972								
No. cuttings	22	22	0	26	0	0	0	70
No. rooted	11	10	0	12	0	0	0	33
% rooted	50.0	54.4	0	46.1	0	0	0	47.1

Very few of the cuttings taken in early June rooted (9%) and the female cones died, probably because the material was very soft and rotted on the mist bench. Bogdanov (1984), found Picea abies cuttings rooted best in late July in Bulgaria. It is possible that P. contorta cuttings may have rooted more successfully if they had been taken a few weeks later, when the material was less soft.

The cuttings taken in October were the most successful. Between 45-52% of the cuttings of clone 8972 rooted and 'held' their cones. However, none of the cuttings of clone 8971 root. Material from tree 8971 had not been propagated before and may be a poor rooting clone.

As only a few cuttings of clone 8972 rooted they were manipulated in several different ways during 1985 to establish the best after-care procedure. The plants were potted into 0.23 litre pots on 24/1/85 and 12/2/85 and left on the mist bench until 4/3/85 but with the frequency and duration of the mist reduced. They were potted into 1.5 litre pots and transfered to another glasshouse (ambient temperature 8 °C to 18 °C, 20 h supplementary lighting with 400 watt MBFR/U lamps). Half the plants of each type (i.e. UF, UV, MV) were transferred to a coldroom (4 °C) for four weeks (15/3/85 to 15/4/85). All the plants were transferred to a frost free glasshouse on the 15/4/85 and appeared to grow normally. As normal vegetative growth and cone development occurred on both sets of plants, it was concluded that chilling was unnecessary. Fourteen female cones were collected in October-November and they shed their seed normally. Following a period of stratification three seedlings germinated. The fully developed cones were, however, smaller (mean length of 20 mm and diameter 16 mm) than similar aged cones on the parent tree (mean length 48 mm and diameter 25 mm). This reduction in cone size may result from only one age class of needles suppling the assimilates during the early growth of the cone. This hypothesis was investigated in 1986, using bark-ringed branches on field grown trees (section 6.6).

In conclusion, it appears that cuttings taken in October root reasonably well, develop normal vegetative and reproductive growth and therefore would be suitable material to use to construct a complete carbon budget of P. contorta plants with and without cones.

6.4.4 SUMMARY

1) Cuttings of P. contorta were taken at three times of the year: (i) in the spring before the female cones were pollinated, (ii) in the summer when the female cones had been naturally pollinated, and

(iii) in the autumn when the female cones had been pollinated and the male cone-bearing buds could be identified.

2) Only the cuttings taken in the autumn were successful, with around 50% of one clone rooting and producing normal vegetative and cone growth.

3) It was concluded that although there appeared to be a reduction in the growth of the cones and possibly the vegetative shoots on cutting taken in the autumn, these cuttings could be used to construct a complete carbon budget for P. contorta plants with and without male and female cones.

6.5 ASSESSING POTTING MEDIA

The vegetative and reproductive cuttings taken in October 1984 appeared to be suitable to construct a complete carbon budget for reproductive structure of P. contorta. Five potting media were therefore tested to determine the most suitable for: (i) growth and development of the vegetative shoot, cone, and roots and (ii) the ease of root extraction from the media.

6.5.1 MATERIALS

Cuttings used in this study were part of the yearly production of P. contorta cuttings taken by the 'Temperate tree improvement' project at I.T.E. They were considered suitable material as they were propagated under the same conditions as the vegetative and reproductive cuttings and were from the same plots of trees used in section 6.4. A total of 2207 cuttings were taken on 27 and 28/3/84 by the same method described in section 6.4. Cuttings from five clones were selected which had rooted well (clone 8021, 8974, 8975, 8985, 8994 see Table 6.2 for details).

6.5.2 METHODS

The rooted cuttings were potted into five different media between the 30/7/84 and the 14/9/84. In addition to the standard peat, loam

and grit based compost used by the 'Temperate tree improvement project' at I.T.E. three other peat based media were tested. To facilitate the recovery of clean roots when the plants were harvested, the peat was sieved through a 0.5 cm sieve and mixed with fine sand, grit or Vermiculite. A set of plants were also potted into an inert volcanic media Vermiperl, after it had been washed through with water to remove the fine dust.

The composts were made as follows:

NC - the standard I.T.E. compost used by the 'Temperate tree cone induction project', 80 litres medium sphagnum peat (Bulrush, Bord-va-Mona, Ireland), 20 liters grit (3-5 mm grit, from Douglas Muir Quarry, supplied by Tilcon Ltd., Glasgow), 20 liters sterilised loam (R. Ballantyne, Gorebridge, Scotland) and 400 g Vitax Q4 compound fertiliser (Vitax Ltd., Burscough, U.K.).

SP - 3 parts sand (grade AB/F, R. Wilson (Plant Hire Ltd.), Denny, Glasgow), and 1 part sieved peat.

GP - 3 parts grit and 1 part sieved peat.

VP - 2 parts Vermiculite (medium grade Silvaperl Vermiculite, Lawson & Donaldson Seed Ltd., Edinburgh) and 1 part sieved peat

VS - washed Vermiperl (medium grade, Silvaperl Vermiperl, Lawson & Donaldson Seed Ltd., Edinburgh).

An acidic fertiliser solution was used when watering the plants potted into VS because slightly acidic condition are recommended for pine (Duryea & Landis 1984) and Vermiperl, because it is inert, assumes the pH of the glasshouse water, which was alkaline (pH of 7.5 to 8.5, EIL 7055 Kent, Electronic Instruments Ltd. Chertsey, Surrey, England). A nutrient solution recommended by Ingestad (1979) for P. sylvestris was acidified with acetic acid to give the required pH (D. Deans pers. comm.). The other four media were watered weekly with the standard I.T.E. soluble fertiliser (Sangral SS20, L & K Fertilisers Ltd., Lincoln, England).

The cuttings were potted directly from the mist bench into 1.5 litre pots rather that the normal 0.23 liter pot. This was because most of the plants had large well formed root systems which would have required to be potted on during the course of this trial.

The survival and growth of the plants in the different media was assessed on 26/7/85 when the shoot growth had ceased. The roots were examined on the healthiest individual of each clone in each treatment on 24/1/84 and in clones 8021, 8985 and 8994 on 6/5/85. The roots were washed and scored on a scale of 0-10 for the quantity of root, ease of cleaning and quality of the cleaned roots.

6.5.3 RESULTS AND DISCUSSION

The sand/peat compost had the highest survival rate while the standard compost had the lowest (Table 6.4). The poor survival and root formation (Table 6.5) of the standard compost was possibly the result of poor drainage and water-logging. In addition the roots from the standard compost were the hardest to clean. The peat incorporated into the normal compost was not sieved and the living roots were difficult to disentangle from the larger strands of peat.

Table 6.4 Number of plants potted into each of five potting media on, and the number and percentage surviving in January and May of the following year, (NC = normal compost, SP = sand/peat, GP = grit/peat, VP = Vermiculite/peat,V = Vermiperl, all clones potted into Vermiperl media on 14/9/84).

Clone	Number of reps	Date of potting	Number surviving on 24th January 1984					Number surviving on 6th May 1985				
			NC	SP	GP	VP	V	NC	SP	GP	VP	V
8021	20	6/8/84	10	20	16	12	10	2	12	9	8	9
8974	14	13/8/84	1	6	4	2	5	0	4	1	1	4
8975	10	13/8/84	6	7	5	6	9	3	5	5	5	6
8985	15	30/7/84	15	12	14	12	11	15	12	14	14	11
8994	14	30/7/84	10	13	13	10	10	10	13	13	10	8
TOTAL	73		42	58	52	42	45	30	48	42	28	40
% SURVIVAL			57	79	71	57	62	41	66	57	38	55

Table 6.5 Quantity and quality of roots and the ease of cleaning the roots, scored on a 0-10 scale for five clones (Cl.) of P. contorta cuttings potted into five different potting media. Assessed on the 24/1/85 (As. 1) and 6/5/85 (As. 2). The potting media were: NC = normal compost, SP = sand/peat, GP = grit/peat, VP = Vermiculite/peat, V = Vermiperl.

		Quantity of roots					Ease of cleaning roots					Quality of cleaned roots				
Cl.	As.	NC	SP	GP	VP	VS	NC	SP	GP	VP	VS	NC	SP	GP	VP	VS
8021	1	2	9	4	7	6	5	7	7	2	4	5	5	5	4	7
8021	2	4	7	8	5	7	2	6	5	4	5	2	6	6	4	7
8974	1	6	8	7	7	5	4	7	7	6	6	3	4	4	5	5
8975	1	7	8	7	6	5	4	7	7	6	6	3	4	4	5	6
8985	1	9	8	9	8	6	2	7	7	6	6	3	5	5	5	7
8985	2	4	7	8	4	7	5	7	7	2	7	4	5	5	3	7
8994	1	9	8	6	7	6	2	7	7	6	6	5	5	5	5	7
8994	2	6	7	7	4	5	1	7	7	5	5	1	7	7	5	6
Mean	1	6.6	8.2	6.6	7.0	5.6	3.6	7.0	7.0	5.2	5.6	3.8	4.6	4.6	4.8	6.4
Mean	2	4.7	7.0	7.6	5.6	6.3	2.7	6.7	6.3	3.7	5.7	2.3	6.0	6.0	4.0	6.7

Over all the clones, the plants growing in the standard compost had the longest terminal shoot at the end of the 1985 growing season (Figure 6.6a), while the plants potted into Vermiperl had the shortest shoots. There was, however, considerable clonal variation in shoot growth (Figure 6.6 b-f). The plants potted into Vermiperl looked yellow a few months after potting. This was attributed to spraying with the acidic Ingestad solution. Thereafter the solution was watered onto the compost. It was evident when the roots were examined in January that they appeared to be recovering from a 'check' by producing a lot of new healthy roots. The new root growth probably correlated with the improved fertiliser application. Despite the discouraging early growth, Vermiperl was considered a good medium for P. contorta cuttings with the advantage that its inert nature would allow easier measurement of root respiration. It was decided therefore to use either sand/peat mixture or Vermiperl in future trials.

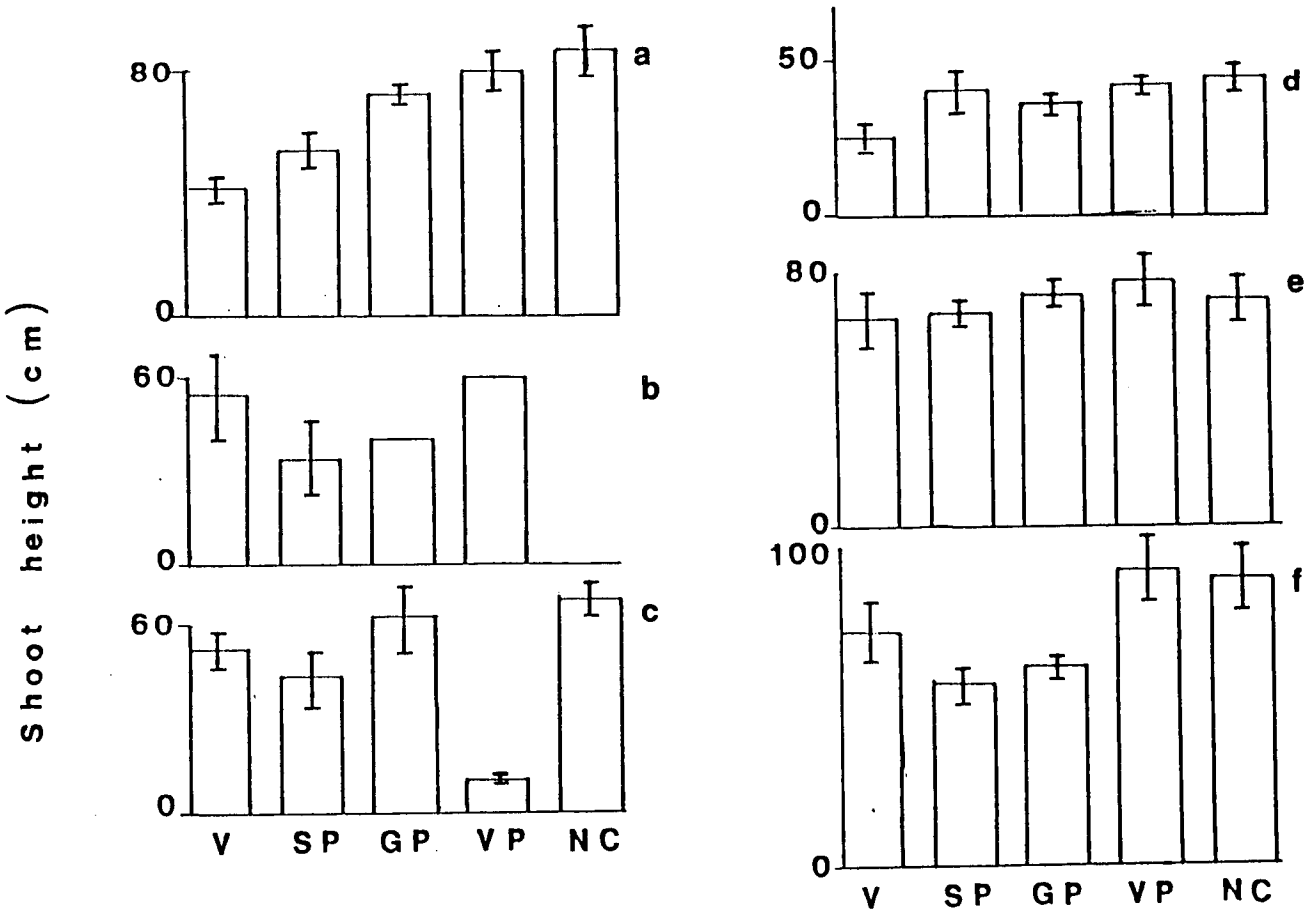


Figure 6.6 Mean height of the terminal shoot of a) five clones, b) clone 8974, c) 8975, d) 8021, e) 8994, and f) 8985 of P. contorta plants growing in five media, V=vermiperl, SP=sand/peat, GP=grit/peat, VP= vermiculite/peat and NC=standard compost.

6.5.4 SUMMARY

1) Five media were tested to determine the most suitable for the rooted cuttings of P. contorta. The media tested were: (i) standard I.T.E. compost, (ii) sand/peat, (iii) grit/peat, (iv) Vermiculite/peat, (v) Vermiperl.

2) Cuttings growing in the standard compost had the longest terminal shoot but the worst root growth and low percentage survival. The sand/peat and the grit/peat were both good media in terms of shoot and root growth and ease of cleaning the roots.

3) The plants potted in Vermiperl initially grew poorly, but an improved technique for applying the acidic nutrient solution resulted in good shoot and root growth. Because Vermiperl is inert it should be easier to obtain root respiration measurements for plants potted in this media.

4) It was concluded that either a sand/peat mixture or Vermiperl would be suitable media to use when constructing a complete carbon budget of P. contorta cuttings with and without cones.

6.6 EFFECT OF ONLY TWO AGE CLASS OF NEEDLES ON FEMALE CONE GROWTH

The early results from the vegetative propagation of reproductive cuttings (section 6.4) indicated that female cones were smaller when they developed on cuttings with only two age classes of needles. The aim of this trial was to determine whether restricting assimilate importation to only two age classes of needles affected the growth and development of female cones on field-grown trees.

6.6.1 MATERIALS

Three trees were chosen in mid May 1986 in the 'Nursery' plot of I.T.E. at the Bush Estate, near Edinburgh. All three trees had been grown from seed in 1970 and planted in 1971 as part of a provenance trial. The original seed for each tree came from a different provenance; (8968 from Takysie, British Columbia, 8988

from Cedarvale, Skeena River, British Columbia, and 8989 from Terrace Creek, British Columbia).

An initial assessment of the number of n2 female cones and the length of the previous year's shoot i.e. the parent shoot, was recorded on 125 branches. Branches with the same number of n2 female cones and similar length of parent shoot were considered a 'pair' and one was randomly assigned to each treatment by tossing a coin. Unfortunately, as the result of bark-ringing, several branches broke during the summer. These and their 'non-ringed pair' were therefore excluded from further analysis. A total of 32 'paired' branches survived to the end of shoot extension (Table 6.6), but only 22 still remained in October when the cones were collected.

Table 6.6 Number of 'paired' branches originally chosen, one pair of which was bark-ringed at the base of the parent shoot in May 1986 on three trees of P. contorta, and the number of branches which survived to the end of June and until mid October when the cones were collected.

	Number of paired branches			
Tree	Originally chosen	Surviving at the end of June	Surviving by mid October	Percentage of branches broken during the summer
8986	11	11	10	9
8988	12	8	5	58
8989	18	13	7	61
Total	41	32	22	46

6.6.2 METHODS

A 5 mm section of bark was removed, using the 'I.T.E. ringing tool' fitted with number 12 scalpel blades (Swann Morton LTD. Sheffield, England). The 'ITE ringing tool' consisted of two scalpel handles cut to a length of 7 cm and joined together by threaded rod. The

width of the ring was set by adjusting screws on the threaded rod. The bark was removed at the base of the parent shoot on the 19/5/86 and the wound sealed with a pruning compound ('Arbrex', Pan Britannica Industries Ltd., Waltham Cross, Hertford, England).

The length of the terminal shoot and the length and diameter of the n2 female cones were measured during the summer with a ruler and calibers (Batty model, Mackay and Lynn, Edinburgh).

Although considerable care was taken to ensure that the branches were 'paired' by the length of the parent shoot and the number of n2 female cones it was not possible to similarly pair for the number of n1 female cones as they were enclosed in bud scales. Therefore the n1 female cones were removed when they emerged because they would have distrubted the orginal pairing. A total of 14 branches each of the ringed and the non-ringed treatments had n1 female cones. These were cut off with a scalpel on 25/6/86 (20 from bark-ringed branches and 28 from the non-ringed branches). The n2 female cones were collected from all the trees on 6/10/86 (Table 6.7). They were left in the laboratory in paper bags to open and shed their seed. Cones which had not opened their scales by 5/11/86 were dipped in boiling water for 20-25 seconds and placed in an oven at 35-40 °C This method is used by the Forestry Commission to stimulate cone opening (A Fletcher pers. comm.). The germination percentage was determined based on the method described in the International Rules for Seed Testing (Annon 1959). As a result of limited seed number groups of 50 seed were placed on moist filter paper in 9 cm petri dishes and stored in a cold room (4 °C) for 21 days. The number of replicates varied with clone and treatment depending on the number of seed available (Table 6.7). The petri dishes were transfered, after stratification, to an unlit Fison growth cabinet (FI-TOTRON 600H, Fison Environmental Equipment, Loughborough, England) set at 20 °C for 16 hours and 30 °C for 8 hours. The number of seeds germinating at 7 day intervals was recorded and the percentage of filled seed germinated by day 21 was calculated.

Table 6.7 Number and mean dry weight of n2 female cones, number and mean dry weight of seed and the percentage of filled seed germinated from three trees of P. contorta, treated in May 1986. Ring = 5mm ring of bark removed from the base of the parent shoot. Non-ring = not treated.

Tree	8986		8988		8989	
Treatment	non-ring	ring	non-ring	ring	non-ring	ring
Number of n2 female cones	20	20	9	9	18	18
Mean dry weight of n2 female cone*	6.12 (0.29)	4.65 (0.22)	4.24 (0.21)	3.28 (0.16)	3.83 (0.17)	2.52 (0.08)
Mean dry weight of seed (mg)	3.6	3.2	3.3	2.9	2.4	2.0
Total number of seed	534	585	292	402	546	299
Mean number of seed per cone	26.7	29.2	32.4	44.7	30.3	16.6
Percentage seed germination	100	100	99	99	95	94

* Standard error of the mean

6.6.3 RESULTS

The 32 'paired' branches which survived until the end of shoot extension had similar parent shoot length (Figure 6.7) and each pair had exactly the same number of n2 female cones (1-4). Shoot extension of the 1986 terminal shoot had ceased by the end of June in both the bark-ringed and non-ringed branches (Figure 6.8). Branches which had been bark-ringed had significantly shorter terminal shoots at the end of the growing season compared with non-ringed branches. (Table 6.8). Similarly, the n2 female cone ceased to grow in length and diameter by the end of June (Figure 6.9) and treatment significantly reduced both the length and diameter of the n2 female cones (Table 6.8) Treatment also significantly reduced the final dry weight of the n2 female cones collected in October in all three trees (Table 6.7).

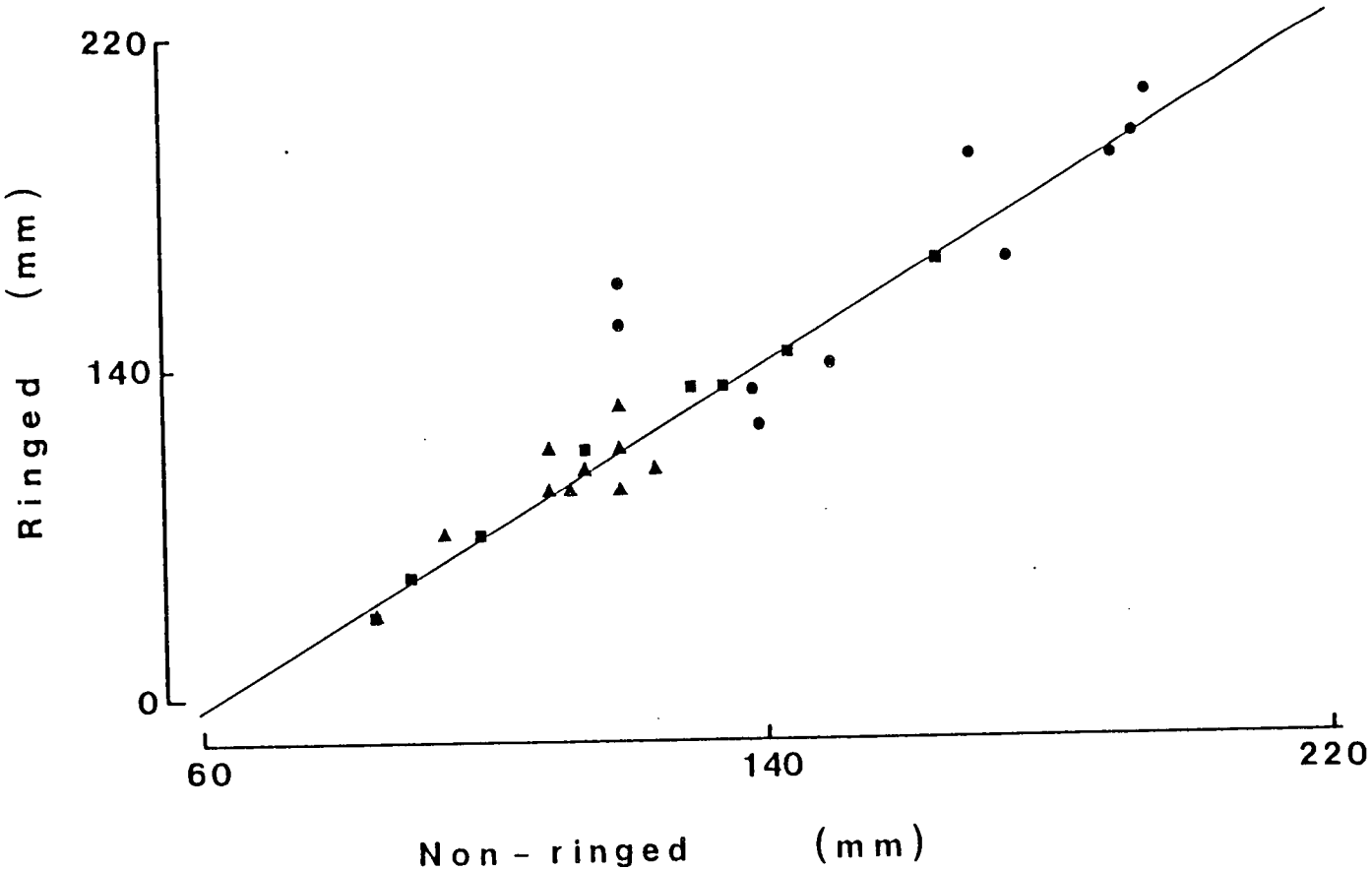


Figure 6.7 The length of the parent shoot of ringed and non-ringed branches of three P. contorta trees(● 8986, ■ 8988, ▲ 8989).

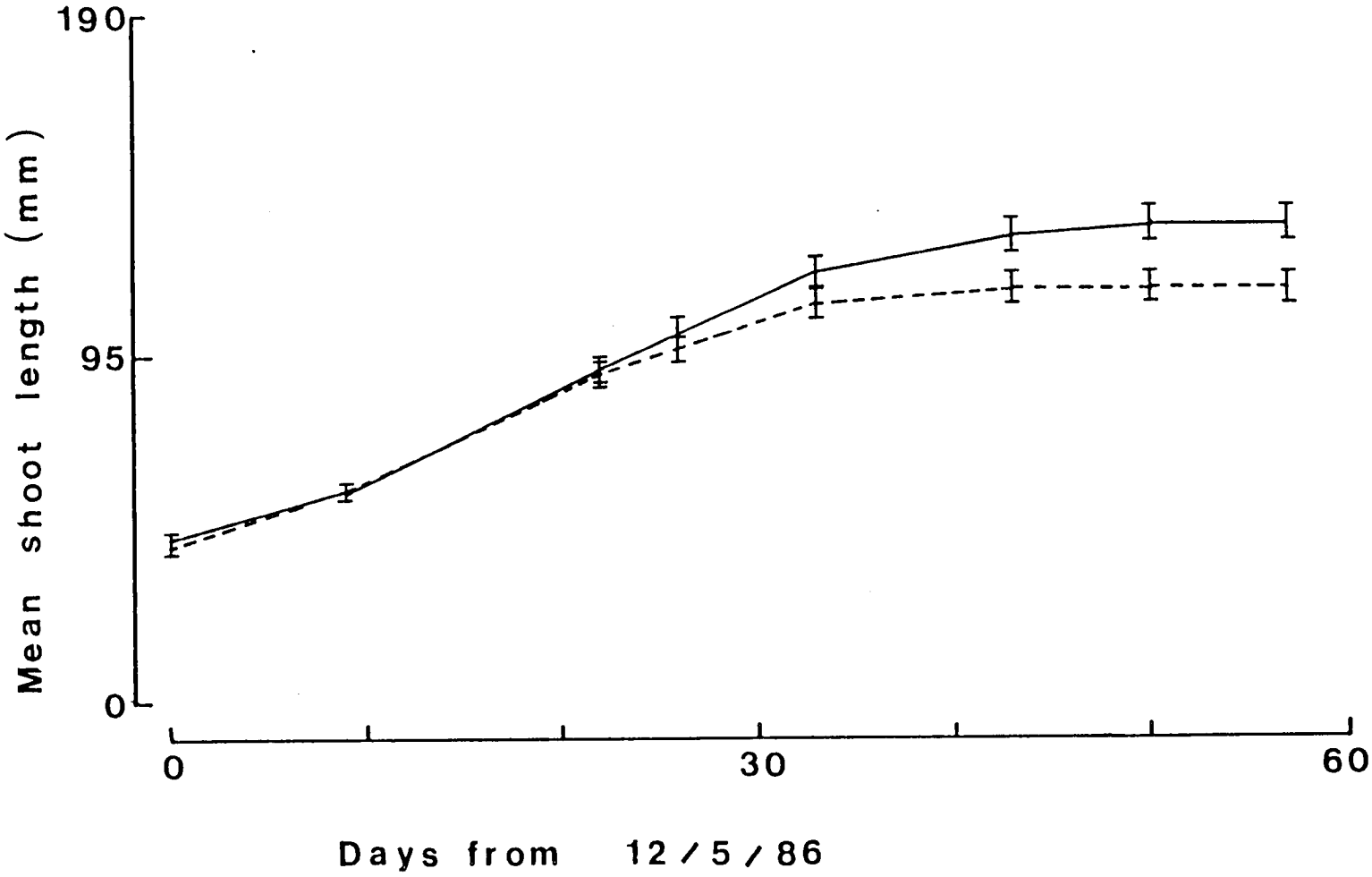


Figure 6.8 Mean length of terminal shoot of the current year's growth on --- ringed (5 mm) and —— non-ringed branches on three trees of P. contorta.

Table 6.8 Analysis of variance table of the: (i) final shoot extension (ii) mean length and (iii) mean diameter of n2 female cones and (iv) the dry weight of n2 female cones on paired branches of P. contorta on three trees. Half the branches had been subjected to a bark-ringing treatment.

Source of variation	DF	Sum of Squares	Mean sum of Squares	F Value	P Value
(i) Terminal shoot extension					
Tree	2	14783	73915	28.55	< 0.001
Branch pair	29	17202	593	2.29	< 0.05
Treatment	1	4970	4970	19.20	< 0.001
Tree * Treatment	2	90	45	0.17	ns
Residual	29	7508	259		
(ii) Mean length of n2 female cones per branch					
Tree	2	634.0	317.0	117.30	< 0.001
Branch pair	19	178.1	9.4	3.47	< 0.01
Treatment	1	101.0	101.0	37.40	< 0.001
Tree * Treatment	2	1.5	0.7	0.28	ns
Residual	19	966.0	50.8		
(iii) Mean diameter of n2 female cones per branch					
Tree	2	189.6	94.8	156.20	< 0.001
Branch pair	19	29.1	1.5	2.52	< 0.05
Treatment	1	10.8	10.8	17.84	< 0.001
Tree * Treatment	2	0.3	0.1	0.28	ns
Residual	19	11.5	0.6		
(iv) Mean dry weight of n2 female cones per branch					
Tree	2	42.7	21.3	64.52	< 0.001
Branch pair	19	13.1	0.1	2.08	ns
Treatment	1	15.7	15.7	47.51	< 0.001
Tree * Treatment	2	0.4	0.2	0.61	ns
Residual	19	78.3	4.1		

The bark-ringed trees produced slightly lighter seeds (Table 6.7). However, there was little difference in the percentage of filled seed which germinated (Table 6.7).

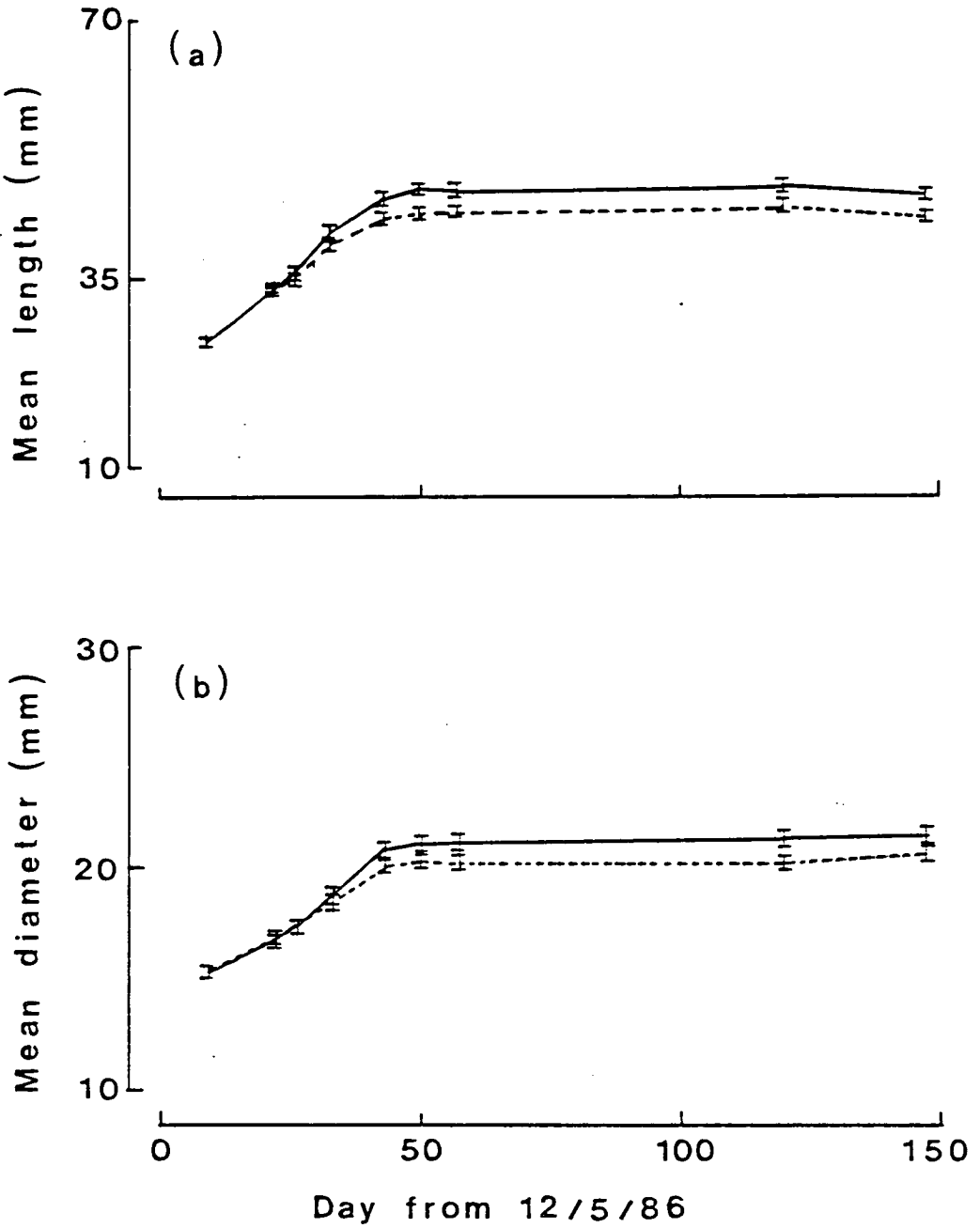


Figure 6.9 a) Mean length and b) mean diameter of n2 female cones on --- ringed (5 mm) and —— non-ringed branches on three P. contorta trees.

6.6.4 DISCUSSION

Terminal shoot extension ceased about the end of June on both bark-ringed and non-ringed branches. Although the initial growth rate was similar for both sets of branches the final length of the terminal shoot on bark-ringed branches was reduced by 13% compared with non-ringed branches. The treatment (5 mm strip of bark removed from the base of the parent shoot) was not applied until mid May when the shoots had already extended by an estimated 35% of there final length. It is probable thefore that if the bark-ringing had been done earlier a larger difference in the extension of the terminal shoot may have occurred. The small reduction in terminal shoot extension indicates that the parent shoot supplied the major proportion of assimilates for the growth of the terminal shoot. Similar results have been found by other workers (see review in Ericsson 1980).

Both the length and the diameter of the n2 female cones growing on bark-ringed branches were also reduced, but to a lesser extent (7%) than the terminal shoot growth. This result is consistent with the evidence from ^{14}C studies (Dickmann & Kozlowski 1970a) that female cones are the most powerful sink for current assimilates. The dry weight of the n2 female cones was reduced by approximately 27% on branches which had been bark-ringed. However the percentage of filled seed germinating after 21 days was the same for both bark-ringed and non-ringed branches. This indicates that the reduction in cone weight did not reduce the viability of the seed. It is not possible to determine the vigour of the seedlings produced on bark-ringed branches as the seedlings were not grown on in this study.

The number of seeds per cone varied inconsistently with treatment, probably because the n2 female cones had already been pollinated prior to treatment.

It is concluded that, branches and probably reproductive cuttings with two age classes of needles will result in both reduced vegetative growth and a reduction in the growth and weight of the

reproductive structures. However, as both vegetative cuttings and the reproductive cuttings would have similar photosynthetic potential it is considered valid to investigate the influence of reproductive structures in this manner.

6.6.5 SUMMARY

1) Branches with a 5 mm ring of bark removed from the base of the parent shoot in mid May had 13% less terminal shoot growth by the end of the growing season. The length and diameter of n2 female cones growing on the bark-ringed branches was also reduced (7%) along with the dry weight (27%) of the n2 female cone.

2) There was also a slight reduction in the weight of seed produced in the n2 female cones on the bark-ringed branches, however, there was no reduction in the percentage of filled seed which germinated.

3) It is concluded from this study, that although there is probably importation of assimilates into growing branches, a system with only two age classes of needles would give a good working approximation, upon which to construct a carbon budget for P. contorta trees with and without cones.

6.7 CUTTINGS TAKEN IN 1985

In the light of the encouraging results obtained with cuttings taken in October 1984, 1126 similar cuttings were collected in October 1985. In addition, a bark-ring or girdle was cut as a preseverence treatment on two clones to encourge quicker development of a root system and increase the chances of cone retention and development. Thulin & Faulds (1968) have found this treatment enhanced rooting of Pinus radiata cuttings. Cameron and Thomson (1969) suggested that preseverence girdling was effective because callus formation is a prerequisite for rooting. Cameron (1970) went on to show that girdling also resulted in the accumulation of assimilates at the site of root initiation, which he suggested enhanced rooting.

6.7.1 MATERIALS AND METHODS

A total of 89 branches on two trees were bark-ringed or girdled at the base of the parent shoot on 23/8/85 (Table 6.9). A 5 mm section of bark was removed, using the 'I.T.E. ringing tool'. The wound was painted with 2 g commercial rooting powder 'Strike', mixed with 10 cm^3 water and sealed with a pruning compound 'Arbrex'. The bark-ringed branches, along with similar non-ringed branches from the same region of the tree crown, were taken as cuttings on the 8/10/85. They were treated in the same way as the cuttings taken in 1984 (see section 6.5.2)

Table 6.9 Number of vegetative and reproductive cuttings of P. contorta taken from non-ringed and bark-ringed (5 mm 23/8/85) branches on the 8th October 1985 and the number and percentage of cuttings rooted by the 16th May 1986. Type of cutting was coded as follows V=vegetative, F=female cone, M=male cone and U=unidentified bud, the first letter describes the bud followed by the type of parent shoot.

	Non-ringed branches				Bark-ringed branches			
Clone	UF	MV	VV	TOTAL	UF	MV	VV	TOTAL
8969								
No. cuttings	17	16	2	35	16	15	0	31
No. rooted	0	0	1	1	0	0	0	0
% rooted	0	0	0	2.8	0	0	0	0
8971								
No. cuttings	20	34	6	60	19	36	3	58
No. rooted	2	6	3	11	16	23	2	42
% rooted	10.00	17.6	50.0	18.3	84.2	63.8	66.7	72.4

In addition to the bark-ringed and control branches, a total of 1126 branches from 9 trees were collected and struck as cuttings in the same way as the cuttings taken in October 1985 (Table 6.10 and Table 6.2).

Table 6.10 Number of vegetative and reproductive cuttings taken from P. contorta trees between 8th and 9th October 1985 and the number and percentage of cuttings rooted by the 29th May 1986. Type of cutting was coded as follows V=vegetative, F=female cone, M=male cone and U=unidentified, the first letter describes the bud followed by the type of parent shoot.

Clone	UF	UM	UV	MV	MM	MF	VV	TOTAL
8964								
No. cuttings	25	62	96	0	0	0	0	183
No. rooted	0	8	36	0	0	0	0	44
% rooted	0	12.9	38	0	0	0	0	41.6
8965								
No. cuttings	17	0	0	30	22	0	13	82
No. rooted	2	0	0	6	6	0	7	21
% rooted	11.7	0	0	20.0	27.2	0	53.8	25.6
8966								
No. cuttings	45	0	0	38	36	0	0	119
No. rooted	16	0	0	28	26	0	0	76
% rooted	35.5	0	0	73.6	72.2	0	0	63.8
8967								
No. cuttings	43	31	59	15	28	0	0	176
No. rooted	0	0	0	0	0	0	0	0
% rooted	0	0	0	0	0	0	0	0
8968								
No. cuttings	28	0	41	34	0	0	0	103
No. rooted	14	0	9	16	0	0	0	39
% rooted	50.0	0	21.9	47.0	0	0	0	37.9
8969								
No. cuttings	23	0	2	26	64	0	0	115
No. rooted	2	0	0	0	1	0	0	3
% rooted	8.7	0	0	0	1.5	0	0	2.6
8971								
No. cuttings	19	17	0	16	0	0	0	52
No. rooted	5	3	0	2	0	0	0	10
% rooted	26.3	17.6	0	12.5	0	0	0	19.2
8972								
No. cuttings	53	54	0	78	46	0	0	231
No. rooted	2	11	0	8	3	0	0	24
% rooted	3.7	20.4	0	10.2	6.5	0	0	10.4
8983								
No. cuttings	21	0	21	78	46	0	0	241
No. rooted	0	0	2	8	3	0	0	8
% rooted	0	0	9.5	10.2	6.5	0	0	3.3

6.7.2 RESULTS AND DISCUSSION

Unfortunately an electrical failure between 25/11/1985 and 19/12/1985 resulted in the house temperature falling to 0 °C and fluctuating with ambient between 3-9 °C (Figure 6.10). While this did not kill the cuttings it severely delayed the rooting process and they did not root for six months. Overall only 20% of the cuttings rooted. However, they were very poor plants and neither the cones nor the shoots grew well during the year. As the material was so poor it was not considered valid to attempt to construct a carbon budget for the vegetative and reproductive branches.

Overall, 72% of the bark-ringed cuttings of clone 8971 rooted compared with 18% of the non-ringed control branches (Table 6.9). However none of the bark-ringed cuttings of clone 8969 rooted and only one of the non-ringed cuttings rooted. These results while agreeing with those of Thulin & Faulds (1968) are not conclusive.

As increased bed temperature hasten root development (Cameron & Rook 1974) it was decided to take more cuttings in 1986 and to use the underbed heating coils to promote the rooting process.

6.7.3 SUMMARY

1) Cuttings treated with a bark-ringing preseverence treatment appeared to have a higher rooting percentage compared with non-ringed cuttings. However only one of the two clones responded to treatment.

2) Unfortunately, an electrical failure, resulted in low glasshouse temperatures during propagation and rooting was poor.

3) In conclusion, it would appear that the failure to produce sufficient good quality cuttings was the result of mechanical rather than physiological reason. It was therefore decided to take cuttings again in the autumn of 1986.

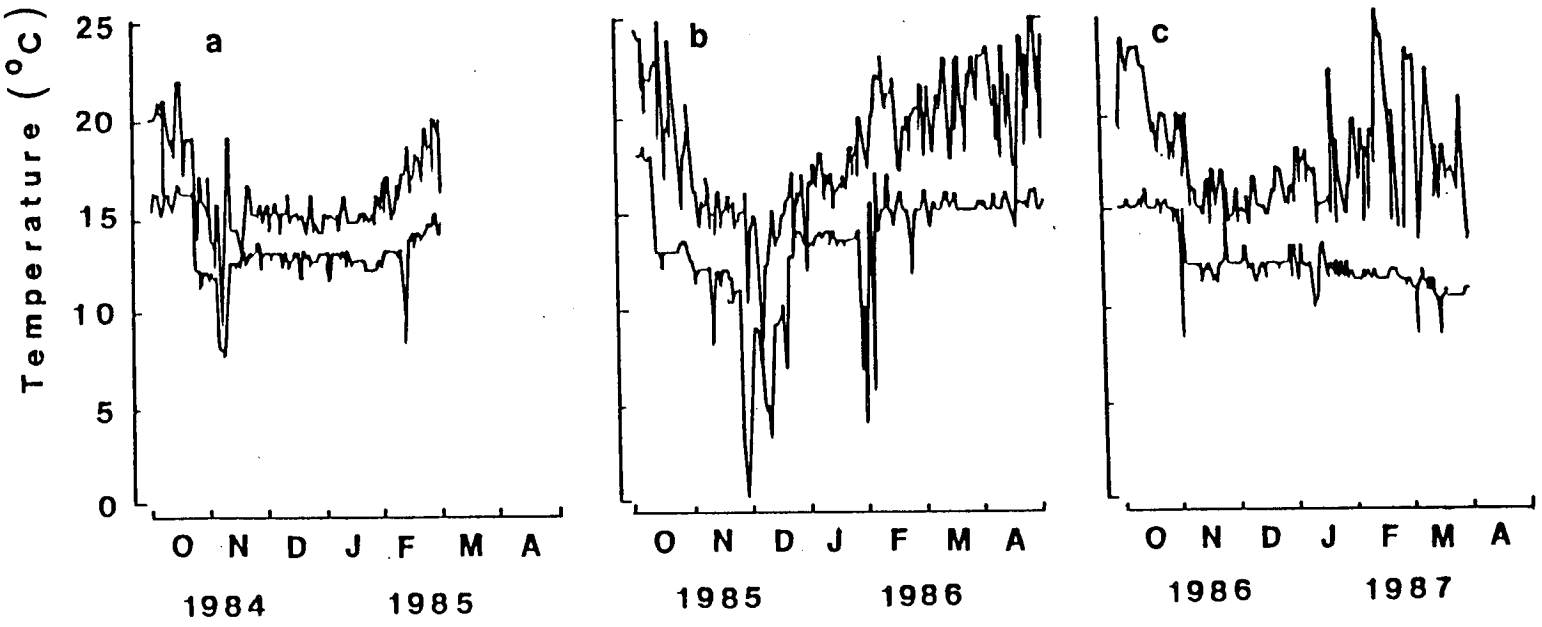


Figure 6.10 Maximum and minimum glasshouse air temperature for three years when cuttings were in the mist benches.

6.8 CUTTINGS TAKEN IN 1986

Because the results of the 1985 propagation were poor a major effort was made to obtain vegetative and reproductive cuttings in 1986.

6.8.1 MATERIAL AND METHODS

A total of 7744 cuttings representing seven types were taken from four clones 8001, 8004, 8020 and 8021 (Table 6.2) between 15-29/10/86 (Table 6.11). In addition, 52 cuttings which had been bark-ringed and 68 equivalent non-ringed cuttings of clone 8001 were taken (Table 6.12).

Table 6.11 Number of vegetative and reproductive cuttings taken from P. contorta trees between 15th and 29th October 1986 and the number and percentage of cuttings rooted by the 23rd March 1987. Type of cutting was coded as follows V=vegetative, F=female cone, M=male cone, U=unidentified bud, and P=polycylic bud, the first letter describes the bud followed by the type of parent shoot.

Clone	UF	UM	UV	MV	MM	MF	PV	TOTAL
8001								
No. cuttings	605	41	1145	676	488	2	0	2957
No. rooted	3	0	3	0	1	0	0	7
% rooted	0.5	0	0.3	0	0.2	0	0	0.2
8004								
No. cuttings	340	7	525	448	262	26	73	1681
No. rooted	1	0	17	2	2	0	0	22
% rooted	0.3	0	3.0	0.4	0.8	0	0	1.3
8020								
No. cuttings	289	131	1021	312	186	0	26	1965
No. rooted	2	0	15	0	0	0	1	18
% rooted	0.7	0	1.5	0	0	0	3.8	0.9
8021								
No. cuttings	154	18	196	333	170	0	270	1141
No. rooted	1	0	2	3	1	0	4	11
% rooted	0.6	0	1.0	0.9	0.6	0	1.5	1.0

Table 6.12 Number of vegetative and reproductive cuttings taken from non-ringed and bark-ringed (5 mm 27/6/86) branches on P. contorta trees on the 8th October 1985 and the number and percentage of cuttings rooted by the 23th February 1987. Type of cutting was coded as follows V=vegetative, F=female cone, M=male cone, and U=unidentified bud the first letter describes the bud followed by the type of parent shoot.

	Non-ringed branches				Bark-ringed branches			
Clone	UF	UV	MV	TOTAL	UF	UV	MV	TOTAL
8001								
No. cuttings	17	44	4	68	16	16	1	52
No. rooted	1	0	0	1	1	0	0	1
% rooted	5.9	0	0	1.5	6.2	0	0	1.9

The cuttings were collected and processed by the same procedures as used in 1984 (section 6.5.2). The underbed heating cables were set at 20 °C (Complex soil heating cables controlled by Camplex Electronic Thermostat (Thermoforce Ltd., Chelmsford, U.K.).

Unfortunately, probably as the result of increased bed heat the lower part of the cuttings started to rot. The under bed heating was therefore switched off and all the cuttings were retrimmed and again treated with 'Strike' between the 19 to 23/2/87.

6.8.2 RESULTS AND DISCUSSION

The overall rooting percentage was 0.7% (Table 6.11), which was probably a direct effect of the cuttings rotting early in 1987. Cameron & Rook (1974) found that for P. radiata air and bed temperatures of 20/15 °C (day/night) gave 70% rooting while 25/15 °C resulted in only 57% rooting. They attributed the difference to an increased fungal infection with higher day temperatures. In this study the bed temperature was set at 20 °C and the air temperature fluctuated between 10° and 17 °C in the initial months. It would appear that these temperatures were too high for P. contorta in mist beds.

Forty percent of the branches bark-ringed as a preseverence treatment, broke before the cuttings were collected. These trees were more exposed than the trees treated in 1985 which probably accounts for the high percentage of deaths. Only one of the cuttings which were bark-ringed produced roots (Table 6.12). The overall rooting percentage was so low that the results from this study can not really be considered as an indication of the effectiveness of bark-ringing as a preseverence treatment.

6.8.3 SUMMARY

1) Over 7744 vegetative and reproductive cuttings were collected in October 1986. Unfortunately, there were very few cutting rooted. It was suggested that this was the result of the underbed heating being set too high (20 °C) which encouraged fungal rot.

2) It was concluded from this study that although few cuttings were rooted after the initial success in 1984, the approach is valid and should be followed up.

6.9 GENERAL CONCLUSIONS

The aim of this study was to develop a system which would allow all the parts of cone-bearing and vegetative plants to be measured so that the influence of cones on the carbon economy of P. contorta plants could be calculated. Two approaches were considered: (i) inducing cones on cuttings already rooted and (ii) inducing mature cuttings with cones to root. The former approach was considered unsatisfactory because the treatments used resulted in significant alterations in the carbon economy of the plants (section 6.3). Reproductive cuttings were successfully rooted and were found to grow and develop normally. The reduction in growth on the cuttings was comparable with bark-ringed branches in the field, indicating that the cuttings did not produce abnormal growth. As both the vegetative and reproductive cuttings would be similarly restricted it was decided that this was the best technique to use when studying the carbon economy of potted plants. A mixture of sieved peat and sand or the inert media Veriperl were considered the most

appropriate media to obtain clean healthy roots. Veriperl probably has the additional advantage that because it is inert root respiration measurements should be easier to obtain. Unfortunately, insufficent cuttings were raised during this study to allow the construction of a complete carbon budget as described in section 6.2. However, this was probabley the result of physical rather than physiological problems. It is therefore recommended that this technique should be investigated further.

CHAPTER 7

INFLUENCE OF MALE AND FEMALE CONES ON THE ASSIMILATE PRODUCTION OF A TREE WITHIN A FOREST STAND

7.1 AIM

Previous chapters have determined the influence of cones on the morphology and CO_2 exchange rate of needles on reproductive branches compared with vegetative branches. It was the aim of this study to incorporate the knowledge gained so far and estimate the influence of male and female cones on the photosynthate production of a tree within a forest stand.

7.2 INTRODUCTION

To estimate the influence of male and female cones on the assimilate production and subsequent growth of P. contorta trees, the CO_2 exchange rate and the dry weight increment on branches, needles, stem and cones would need to be measured. It was planned to collect such data on potted plants, however, the attempt failed (see Chapter 6). An alternative approach was therefore adopted. The mathematical model MAESTRO was used to estimate the light penetration through a stand of trees and to calculate the resultant CO_2 assimilation of a target tree (Wang & Jarvis 1989). By altering the parameters of the model it is possible to determine the influence of male and female cones on the photosynthate production of a target tree within a stand.

Female cones apparently do not influence the needle complement (Chapter 2) or the photosynthetic efficiency of the needles on the branches which bear them (Chapter 3). However they contribute to their own carbon economy by refixing a significant proportion of CO_2 (Chapter 5). MAESTRO was used to estimate the total CO_2 assimilated on a target tree three times during the life cycle of the female cones and the model described in Chapter 5 was used to determine the net respiration rate of female cones on the same days. The

influence of female cones on the carbon economy of trees within forest stands was estimated by comparing the amount of CO_2 assimilated by the needles on the tree with the amount of CO_2 lost as a result of respiration by female cones the same day.

The terminal shoots of male cone-bearing branches have on average 33% less needles than equivalent vegetative branches (Chapter 2). This reduction in needle complement might well result in male cone-bearing trees producing less photosynthate than vegetative trees. The reduction in photosynthate production by the male cone-bearing shoot however, may not be significant because the older needles on the parent shoot of male cone-bearing branches have an increased photosynthetic efficiency compared with equivalent needles on vegetative branches (Chapter 3 & 4). MAESTRO was used to determine the likely difference in the CO_2 assimilation of a 'vegetative' target tree and a similar tree with a 33% reduction in the needle complement and a higher quantum efficiency of CO_2 assimilation on the older needles.

7.3 METHODS

MAESTRO is a model of an array of trees of a particular size, spacing and crown dimensions (Wang & Jarvis 1989). The model treats the transfer of beam and diffuse radiation in the visible (PAR), near infra-red (NIR) and thermal wavebands through the crowns of the trees in the array. When the beam is intercepted by a needle, it is scattered and the resulting bi-directional diffuse fluxes contribute to the diffuse radiation within the canopy. Radiation reaching the ground is reflected back upwards and the bi-directional radiation absorbed by needles in different parts of the canopy is calculated. This information is used in routines that calculate carbon dioxide assimilation, stomatal conductance and transpiration of the needles. The distribution of needles is non-random, in that the needles are grouped into crowns and within the crowns the distribution of leaf area density is defined in both the vertical and horizontal directions. Thus MAESTRO can predict the radiation absorption, photosynthesis and transpiration within the crown of an individual tree in a stand. The model can predict the radiation

flux at a particular location within the stand and this has made straightforward validation possible (Wang, 1988).

The following general assumptions have been made in MAESTRO:
(i) The inputs of radiation fluxes, air temperature and relative humidity are hourly average values and are appropriate for calculating hourly and daily radiation absorption, photosynthesis and transpiration of the crown of a tree within a stand of trees.
(ii) The crowns of the trees in the stand are the same geometrical shape (cone, half-ellipsoid or paraboloid), and symmetrical around the tree trunk. The trees were assumed to have a half-ellipsoid shape in this study.
(iii) Needles are continuously distributed within the tree crown. The horizontal and vertical distributions of leaf area density are independent of each other.
(iv) Up to 52 grid points are located within the tree crown, each representing a subvolume of the tree crown The radiation absorption, photosynthesis and transpiration of the tree can be calculated as the weighted sum of the radiation absorption, photosynthesis and transpiration of these grid points: the weighting factor is the total leaf area density within the subvolume.
(v) The air temperature, water vapour pressure deficit of the ambient air and CO_2 concentration profiles are neutral within the canopy,
(vi) There are three different age classes of needles within the tree crown: current, one-year-old and other older needles. The needles within each age class are separated in three ecological types according to their position within the tree crown (upper, middle and lower). Needles with the same age class and ecological type have the same physical and physiological properties.
(vii) Woody parts within the tree crown (tree trunk, twigs and branches) can be ignored for the purposes of simulating radiation transmission, photosynthesis and transpiration of the tree crown.
(viii) PAR is the driving variable for photosynthesis.

MAESTRO is composed of seven independent submodels with the following functions:
(i) calculates position of the sun in the sky during the day and the

daylength,
(ii) partitions the fluxes of radiation into photosynthetic, near-infra red and thermal radiation,
(iii) calculates the leaf area density in volumes in the tree crown,
(iv) calculates the absorbed flux densities of radiation within the tree crown,
(v) calculates the leaf boundary layer, stomatal and mesophyll conductances for each of three age classes and ecological types of needles within a tree,
(vi) estimates transpiration and
(vii) photosynthesis of a target tree within the stand on an hourly and daily basis (24 h), using the photosynthesis submodel (Jarvis et al. 1985) that was used in Chapters 3 & 4 to estimate the assimilation rate /quantum flux density response curves of vegetative, female and male cone-bearing branches.

MAESTRO was used in this study to estimate the net hourly and daily carbon assimilation of vegetative and cone-bearing target trees within two stands of trees with stocking densities of 3690 and 1845 trees ha^{-1}. The structural parameters for all trees in the two stands (Table 7.1) were obtained from published data (Whitehead 1978, Whitehead et al., 1984a,b).

Table 7.1 The morphological characteristics of all the P. contorta trees and the planting density of the stands.

	High Density	Low Density
Number of trees (ha^{-1})	3690	1845
Spacing (m)	1.64	2.33
Age (years)	19	19
Tree height (m)	8	8
Height to base of crown (m)	4	4
Length of crown (m)	4	4
Radius of crown (m)	0.83	0.83
Leaf area (m^2/tree)	15	15

The meteorological data used were collected by Dr A. Crossley and Miss D. Wilson of I.T.E. using a weather station (Precision Model, Didcot Instrument Company Ltd. Abingdon, England) at Glentress forest, Peeblesshire, Scotland (3° 8′ N, 55° 39′ W, 275 m altitude). The physiological parameters estimated in Chapter 3 for needles on vegetative, female and male cone-bearing branches were utilised in the model. Built in default values of physiological parameters appropriate to conifers were used for some parameters which had not been measured, as these appeared to be good estimates for P. contorta (Table 7.2).

7.4 FEMALE CONE-BEARING TREES

7.4.1 METHODS

The model was run to estimate the amount of CO_2 assimilated on a female cone-bearing tree on three kinds of days (sunny, cloudy and intermediate days) at three times of the year:
(i) during the first winter after initiation when the new cone is small and has its highest refixation capacity (7th-9th January 1988),
(ii) in late spring when the cone is growing rapidly and has a high respiration rate (25, 26 & 29th May 1988), and
(iii) in late summer when the cone has ceased to increase in dry weight yet still has a fairly high respiration rate (27th, 28th, and 31 August 1988) (Figure 7.1).

The predicted weight of one cone (see Chapter 5) was 0.24 g in January, 1.38 g to 1.71 g in May and in August the final weight of one cone was 4.52 g.

Female cones are normally found high up in the tree crown, in the regions covered by grid points 10 and 12 (Figure 7.3). The PAR reaching these two grid points (i.e. average daily transmittance of PAR through the canopy to the each grid point) was estimated by MAESTRO (Table 7.3). An average value of 75% transmittance was used in the model described in Chapter 5 to estimate the net respiration

Table 7.2 Physiological parameters used in MAESTRO to estimate the influence of female and male cones on the CO_2 assimilation of a target P. contorta tree with a stand, (g_m = mesophyll conductance, g_s = stomatal conductance, temp = temperature, VPD = vapour pressure deficit, α = quantum flux efficiency (mol mol^{-1}) α_s = spring α_a = autumn, θ = convexity).

Ambient CO_2	350.0 μmol mol^{-1}
Reference height of anemometer	13.0 m
Highest temp. for g_m	40.0 °C
Optimal temp. for g_m	21.5 °C
Lowest temp. for g_m	-5.0 °C
Highest temp. for g_s	40.0 °C
Optimal temp. for g_s	15.0 °C
Lowest temp. for g_s	-5.0 °C
Initial slope of stomatal quantum response curve	0.0204 mol μmol^{-1}
g_s in dark	0.0590 mol m^{-2} s^{-1}
Slope of stomatal response to VPD	0.4037 mol m^{-2} s^{-1} kPa^{-1}
Maximum g_m	0.1000 mol m^{-2} s^{-1}
Maximum g_s	0.4000 mol m^{-2} s^{-1}
Dark respiration at 0 °C	0.2000 μmol m^{-2} s^{-1}
Temp. coefficient for dark respiration	0.082 °C

Age of needle (year)	Ecological type of needle	Female trees			Vegetative trees		Male trees	
		θ	α_s	α_a	θ	α_a	θ	α_a
1	1	0.00	0.03	0.07	0.00	0.06	0.00	0.06
2	1	0.00	0.03	0.07	0.00	0.06	0.39	0.07
3	1	0.00	0.03	0.07	0.00	0.06	0.39	0.07
1	2	0.00	0.03	0.07	0.00	0.06	0.00	0.06
2	2	0.00	0.03	0.07	0.00	0.06	0.39	0.07
3	2	0.00	0.03	0.07	0.00	0.06	0.39	0.07
1	3	0.00	0.03	0.07	0.00	0.06	0.00	0.06
2	3	0.00	0.03	0.07	0.00	0.06	0.39	0.07
3	3	0.00	0.03	0.07	0.00	0.06	0.39	0.07

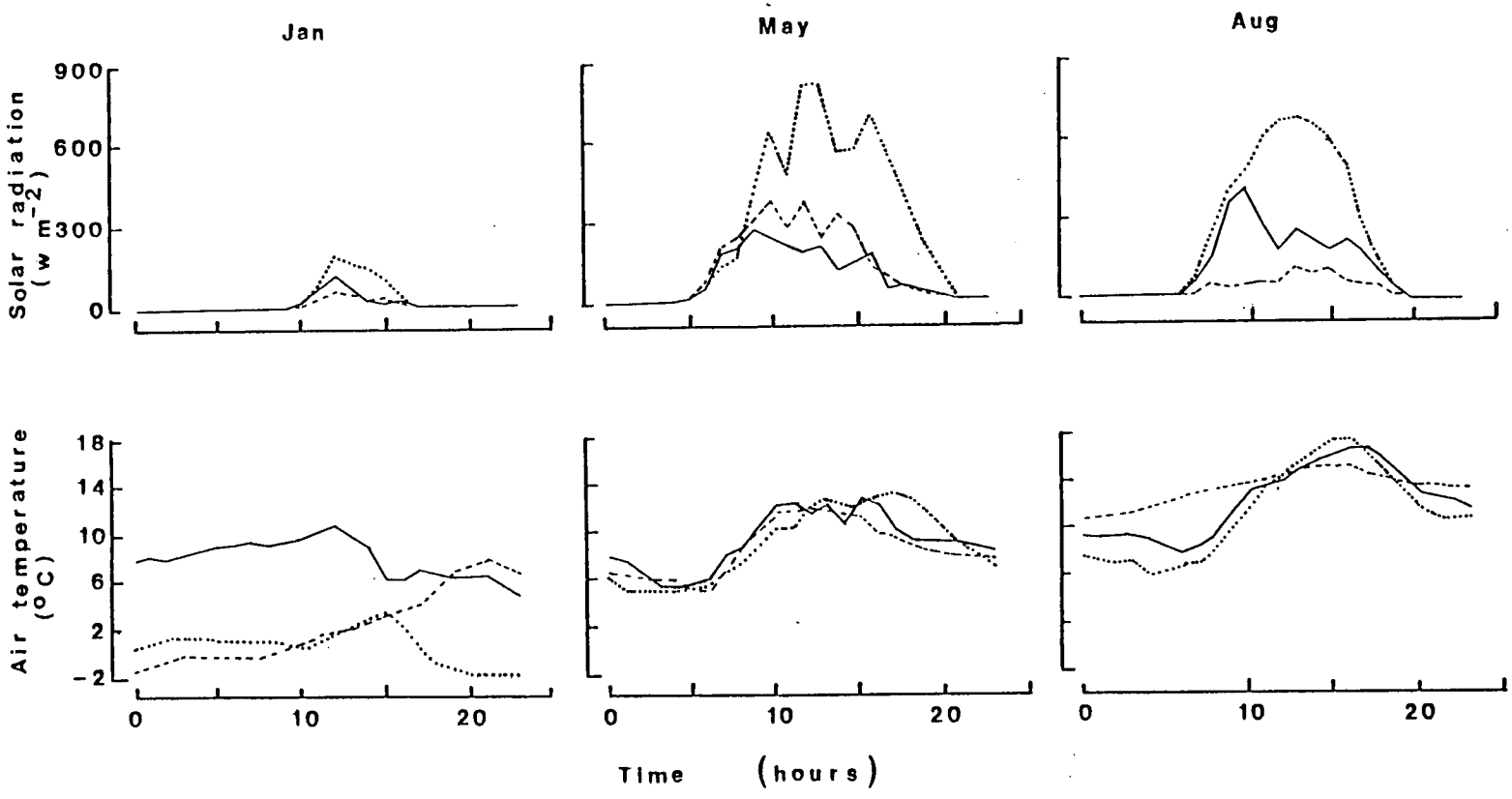


Figure 7.1 Meterological data for three days in January (.... 7th, ---- 8th, —— 9th), May (.... 25th, ---- 26th, —— 29th) and August (.... 27th, ---- 28th, —— 31st) 1988 at Glentress Forest, Peebles used by MAESTRO to estimate the assimilation rate of P. contorta tree.

loss by one female cone for each of the 9 days the MAESTRO model was run. A cone crop of 20 female cones per tree was estimated in the high density stand and 40 female cones per tree in the low density stand (A. Fletcher pers. comm.). The total net respiration loss from the tree was calculated by multiplying the estimated CO_2 efflux for one female cone by 20 or 40 for the high and low density stands, respectively.

7.4.2 RESULTS

On clear sunny days there was up to 20% difference in the average daily transmitted radiation between the 4 quadrants of the target tree at all three sample times, whereas there was very little variation on cloudy overcast days (Table 7.3).

Table 7.3 The average daily transmittance estimated by MAESTRO to grid points 12 and 10 of the target tree in the high and low density stands of P. contorta trees (S=south, E=east, N=north, W=west).

	HIGH DENSITY STAND								LOW DENSITY STAND							
Day	Grid point 12				Grid point 10				Grid point 12				Grid point 10			
	S	E	N	W	S	E	N	W	S	E	N	W	S	E	N	W
7/1/88	73	80	64	85	46	55	45	59	82	85	65	90	59	62	49	72
9/1/88	81	86	77	86	58	64	58	65	85	88	79	87	66	71	62	72
8/1/88	86	86	84	86	64	65	65	65	87	87	86	87	72	71	69	71
25/5/88	90	75	82	88	77	55	56	62	90	75	82	88	70	55	56	62
26/5/88	87	86	84	85	68	60	64	65	87	86	84	85	68	66	63	65
29/5/88	87	86	86	85	66	66	66	64	87	86	84	85	68	66	63	65
27/8/88	92	78	75	89	74	59	45	71	93	79	76	91	85	62	51	78
31/8/88	87	84	84	84	68	65	61	62	88	85	85	86	75	70	66	68
28/8/88	86	85	86	86	65	65	66	65	87	87	87	87	72	71	70	71

Assimilation of the target tree was closely correlated with absorbed PAR at each time of year (Table 7.4). The highest daily assimilation was in late summer on a clear sunny day (27th August), whereas in January there was a negative assimilation balance at the end of the cloudy day. The target tree in the low density stand absorbed more PAR and assimilated more CO_2 than a similar tree in the high density stand.

Table 7.4 The estimated daily PAR absorbed (mol tree^{-1} day^{-1}) by a target tree within: (i) a high and (ii) a low density stand of P. contorta trees on a total of nine days (sunny, moderately sunny and cloudy days) and the amount of CO_2 assimilated by needles on the the target tree and lost from a cone crop of 20 or 40 female cones in the high and low density stands, respectively (mmol day^{-1}).

	Absorbed PAR	CO_2 assimilated Needles on tree	CO_2 assimilated Cone	% of cone/tree assimilation
(i) High density stand				
7/1/88 sunny	11.9	20	-0.32	-1.62
9/1/88 moderate	4.6	-340	-0.06	0.17
8/1/88 cloudy	2.7	-240	-0.41	0.17
25/5/88 sunny	117.6	1960	-5.35	-0.27
26/5/88 moderate	44.2	750	-6.83	-0.91
29/5/88 cloudy	30.6	370	-8.81	-2.38
27/8/88 sunny	95.7	2540	-1.63	-0.06
31/8/88 moderate	39.1	1620	-1.62	-0.10
28/8/88 cloudy	10.1	10	-1.73	-17.26
(ii) Low density stand				
7/1/88 sunny	16.9	130	-0.65	-0.50
9/1/88 moderate	5.9	-290	-1.14	0.39
8/1/88 cloudy	3.2	-220	-0.82	0.37
25/5/88 sunny	151.1	2650	-10.69	-0.40
26/5/88 moderate	54.3	1040	-13.66	-1.31
29/5/88 cloudy	37.6	580	-17.62	-3.04
27/8/88 sunny	133.8	3600	-3.27	-0.09
31/8/88 moderate	50.0	2240	-3.25	-0.14
28/8/88 cloudy	12.5	190	-3.45	-1.82

The CO_2 efflux from female cones was lowest in January. On the sunny day in January, when it was estimated that the trees would have an overall positive carbon balance, the net respiration loss from

female cones was equivalent to 1.6 % and 0.5 % of the CO_2 assimilated by the needles that day in the high and low density stands, respectively. On the moderately sunny and cloud days it was estimated that the trees would have an overall negative carbon balance. However the net respiration losses of CO_2 from female cones was less than 0.5 % of the CO_2 respired by the needles on the tree in both the high and low density stands.

The highest CO_2 efflux of a female cone was estimated for the cloudy day in May. In the high density stand the net respiration loss of CO_2 from 20 female cones was estimated to be 2.4 % of the CO_2 assimilated by the needles of the target tree that day.

The CO_2 efflux of female cones was lower in August than in May, but because the amount of CO_2 assimilated by needles on the tree in the high density stand was very low on the cloudy day it was estimated that the net respiration loss from female cones was equivalent to 17.3% of the CO_2 assimilated by the needles that day. By contrast, because of the increased PAR absorbed in the low density stand, it was estimated that on the same day the net respiration loss from a cone crop of 40 female cones would be equivalent to only 1.8 % of the amount of CO_2 assimilated by the needles that day.

7.4.3 DISCUSSION

The estimated assimilation of CO_2 by needles of the target tree in the three months studied showed the normal seasonal trend found by Troeng & Linder (1982a) for Pinus sylvestris. In January the daylength is short and total solar radiation is low which results in low assimilation and even a negative daily carbon balance. Whereas in the summer and autumn the daylength is longer and the total solar radiation is higher even on cloudy days resulting in more absorbed PAR and consequently a higher daily assimilation. Estimated assimilation was always higher in the low density stand because of the increased PAR absorbed by the individual tree. Vegetative trees would also have the same seasonal trend shown here for a female cone-bearing tree, because needles on vegetative trees did not have a significantly different assimilation rate /quantum flux density

response curve compared with needles on female cone-bearing branches (Chapter 3) and female cones do not reduce the vegetative growth and needle complement of the branches which bear them. Female cone-bearing trees would, however, have a higher total loss of carbon because of the CO_2 respired by the female cone.

The estimated net daily CO_2 efflux of female cones in the three months studied also showed the normal season trends found by Linder and Troeng (1981) for Pinus sylvestris. The net respiration loss of CO_2 from female cones was related to both solar radiation and temperature as defined in Chapter 5. In January the lowest net daily CO_2 respiration loss was estimated on the 7th, probably because it was a relatively cold, sunny day compared to the 8th and 9th. Female cones refix CO_2 during the sunny daylight hours and would respire relatively less CO_2 during darkness because of the lower air temperature. Whereas on the moderately sunny day (9th) air temperature was high and this resulted in larger net daily respiration loss compared with the cloudy day (8th). In May air temperature was similar on all three days studied and the net respiration loss from female cones was closely correlated with the solar radiation presumably because of refixation by the female cone. In August there was very little difference in the net daily respiration loss from female cones between the three days studied. This probably reflects the reduction in both cone respiration rate and refixation capacity as the cones matured.

The net respiration loss from female cones as a proportion of the net CO_2 assimilated by needles on the target tree varied during the three months studied and between the two stand densities. In the high density stand with a cone crop of 20 female cones per tree, it was estimated that on a sunny day in January the female cones lost only 1.6% of the CO_2 assimilated by the needles on the target tree. Despite a larger cone crop (40 female cones per tree) in the low density stand on the sunny day the proportion of CO_2 lost as a result of respiration by the female cones was less (0.50%) compared with the high density stand because of an increase in the net CO_2 assimilated by the needles on the trees in the low density stand. On less sunny days which resulted in a negative assimilation balance by

the needles, female cones contributed a smaller additional burden in the high density stand (0.17%) compared with the low density stand (0.37 and 0.39%) however the CO_2 lost as a result of respiration by the female cones was a small proportion of that assimilated by the needles on the tree in both stand.

In May when the cones had their highest CO_2 efflux the net respiration loss on a sunny day was only a small proportion of the CO_2 assimilated by the needles on the tree (0.27% high density stand and 0.50% in the low density stand). Even on a cloudy overcast day the net respiration loss was not a large proportion of the CO_2 assimilated by the needles on the tree (2.38% in the high density stand and 3.04% in the low density stand). However, it should be remembered that the 'cost' of the cone to the tree in terms of carbon will be higher because it is rapidly increasing in dry weight at this time of year.

In August when the female cones had stopped growing the net CO_2 respiration loss was a small proportion of the needle assimilation on sunny or moderately sunny days in both stand densities ($< 0.15\%$). However in the high density stand on the cloudy day the net respiration loss from female cones was equivalent to 17.26% of the CO_2 assimilated by the needles on the tree that day. In contrast in the low density stand, because of increased PAR absorbed, double the cone crop only resulted in a net respiration loss equivalent to 1.8% of the CO_2 assimilated by the trees on the same day.

This study shows that local weather conditions have a large influence in determining if female cone respiration represents a significant loss of carbon for the tree which bears them. On sunny clear days, when the needles have a large assimilation rate and the female cones refix a large proportion of their respired CO_2, female cones are probably not a significant factor in the growth of the tree. However, the net CO_2 lost in respiration from a substantial crop of female cones may represent a significant loss of carbon for the tree on warm days when the sky is overcast, both because the tree would produce less assimilates and the female cones would have a higher respiration rate and reduced refixation.

In conclusion female cones respire CO_2 and can therefore be considered as a carbon drain on the tree which bears them. However, CO_2 efflux is significantly reduced when the cones are illuminated and their respiration rate is highest when the weather conditions are normally most favourable for photosynthesis. Consequently, the proportion of CO_2 lost as a result of respiration by female cones is probably not large and may be insignificant for the growth of the tree in normal years.

7.5 MALE CONE-BEARING TREES

7.5.1 METHODS

A 33% needle reduction evenly distributed throughout the crown was assumed in the male cone-bearing tree. The model was run three times:

(i) assuming a quantum flux efficiency of all needles equal to that on vegetative branches as given in Chapter 3 and a full needle complement,

(ii) a quantum flux efficiency of all needles equal to that of vegetative needles but with a 33% reduction in needle complement, and

(iii) a quantum flux efficiency of needles on male cone-bearing branches as given in Chapter 3 for the two and three year-old needles and the quantum flux efficiency of vegetative needles for the current year needles, and a 33% reduction in needle complement in all age classes. (Table 7.2).

The model was run for three days in September: a sunny day (22nd), a cloudy day (21st) and intermediate moderately sunny day (23rd September 1987) (Figure 7.2).

7.5.2 RESULTS

The target tree in the low density stand had an estimated higher PAR absorption over 24 h at each grid point compared with the equivalent grid point on the target tree in the high density stand irrespective of weather conditions (Figure 7.3).

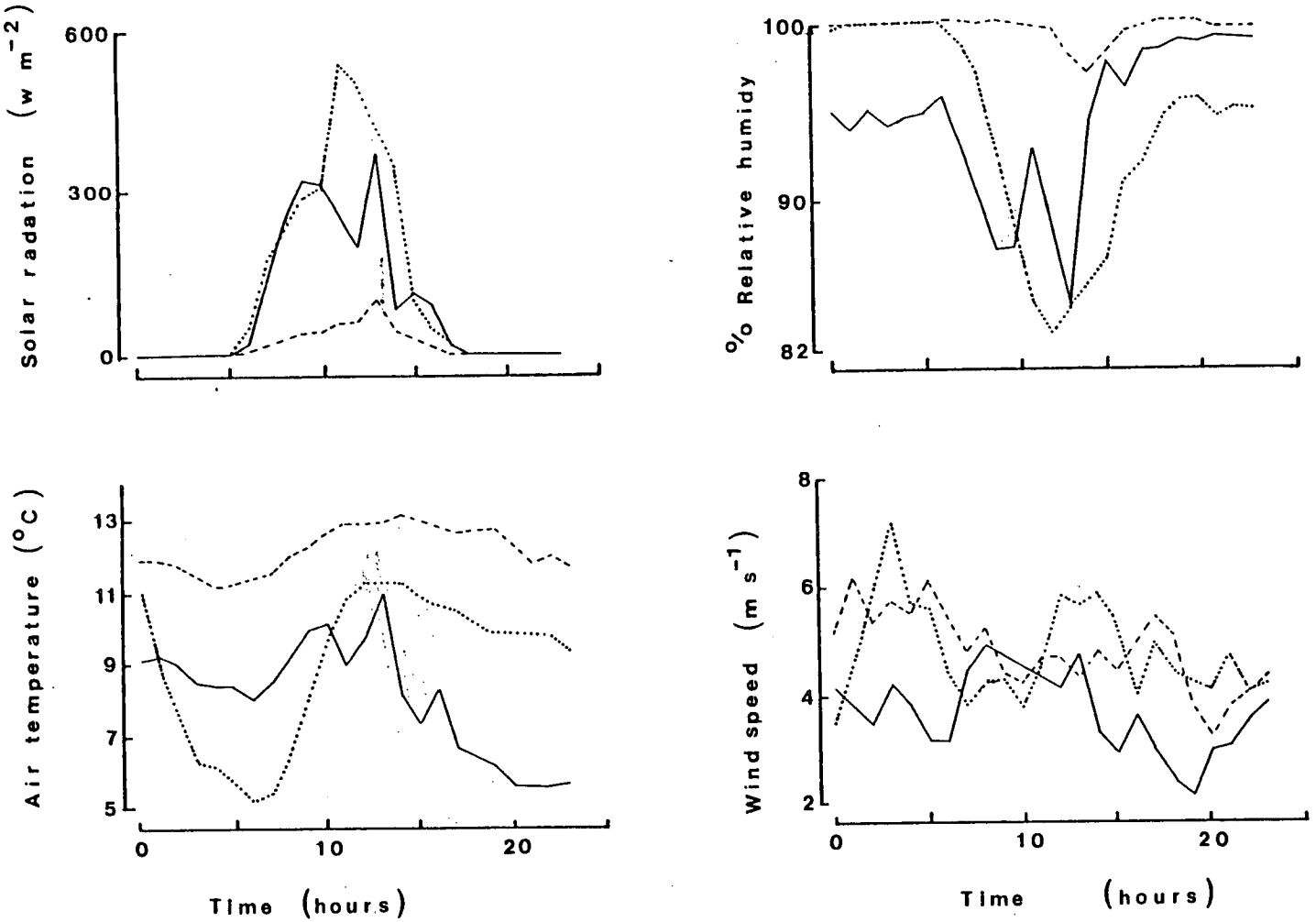


Figure 7.2 Meteorological data for 21st (----), 22nd (....) and 23rd (——) Semptember 1987 at Glentress Forest, Peebles, Peeblesshire, Scotland, used by MAESTRO to estimate the assimialtion of vegetative and male cone-bearing trees of P. contorta.

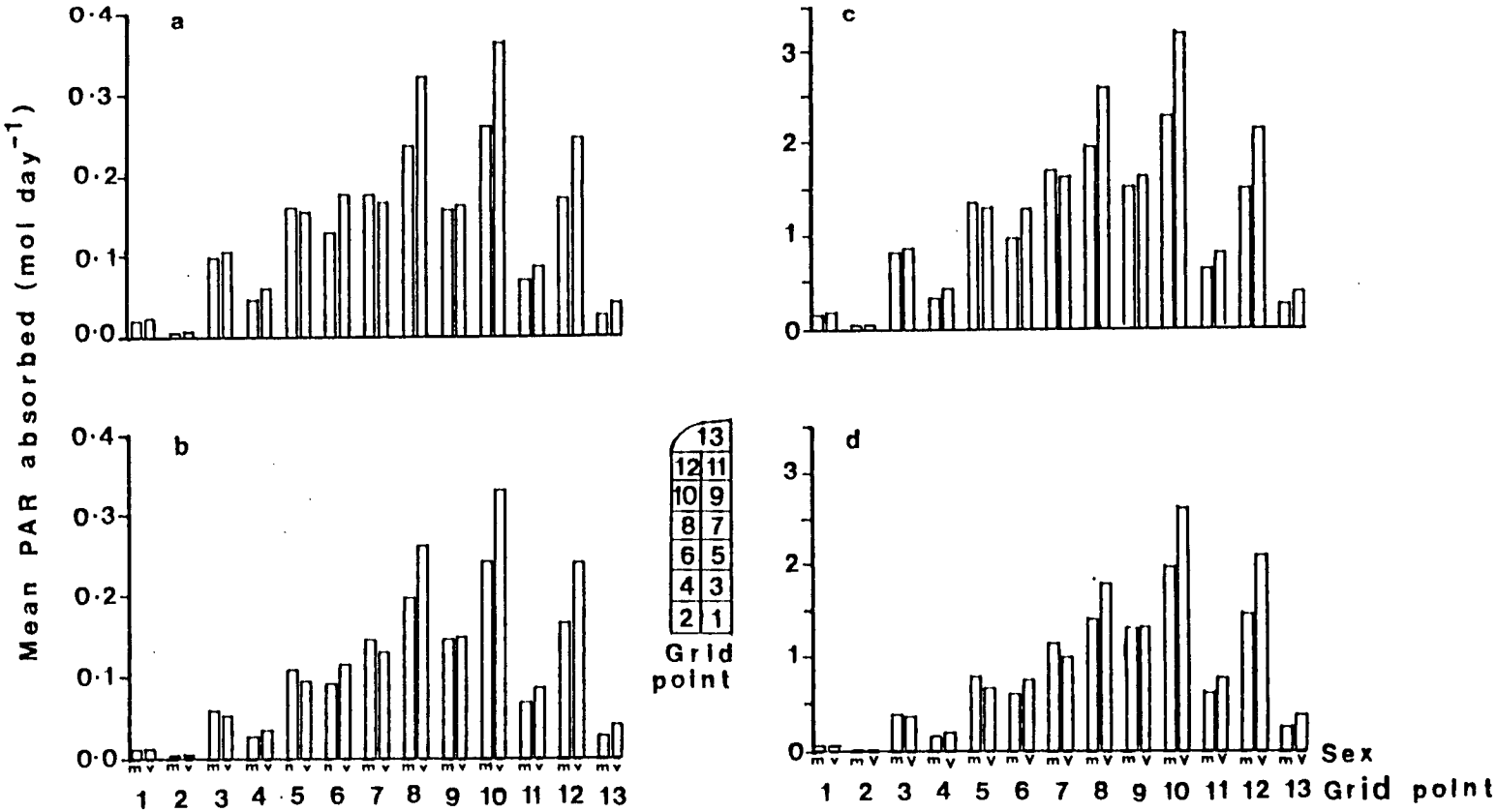


Figure 7.3 The PAR absorbed in 13 grid points (mean of four quadrants), predicted by MAESTRO for a vegetative (v) and male cone-bearing tree on a dull day, 21st September (a & b) and a sunny day, 22nd September 1987 (c & d) in a low density (a & c) and a high density (b & d) stand of P. contorta.

The amount of PAR absorbed was however much more on the sunny day compared with the cloudy day (Figure 7.3).

The PAR absorbed was the same for both vegetative trees with 33% reduction in needle complement and male cone-bearing trees which also had a 33% reduction in needle complement (Table 7.5). Generally the wholly vegetative trees had a larger PAR absorption than the trees with a 33 % reduction in needle complement. However deep within the tree crown at grid points 5 & 7 in the low density stand and grid points 3, 5 & 7 in the high density stand the trees with a 33% reduction in needle complement absorbed more PAR than wholly vegetative trees. (Figure 7.3).

Table 7.5 PAR absorbed and assimilation (A) of CO_2 (mol day^{-1} $tree^{-1}$) on three days in September 1987, a sunny day, moderately sunny and a cloudy day estimated by MAESTRO for (a) a vegetative tree, (b) a vegetative tree with 33% reduction in needle complement and (c) male cone-bearing tree within a: (i) high density and (ii) low density stand of P. contorta trees.

	(a)		(b)		(c)	
	PAR abs	A	PAR abs	A	PAR abs	A
(i) High density stand						
22/9/87 sunny	49.2	1.57	41.7	1.40	41.7	1.58
23/9/87 moderate	32.8	1.18	27.8	1.07	27.9	1.22
21/9/87 cloudy	6.3	-0.30	5.2	-0.14	5.2	-0.10
(ii) Low density stand						
22/9/87 sunny	66.2	2.24	54.4	1.87	54.4	2.10
23/9/87 moderate	44.1	1.72	36.3	1.45	36.3	1.66
21/9/87 cloudy	7.7	-0.21	6.2	-0.07	6.2	-0.03

In the high density stand on a sunny day (22/9/87), a 33% reduction in the needle complement of a vegetative tree resulted in an 11% reduction in the amount of CO_2 assimilated during the 24 hours compared with a wholly vegetative tree (Table 7.5). However the increase in quantum flux efficiency attributed to the two and three-year-old needles on a male cone-bearing tree completely

compensated for the reduction in needle complement. Whereas, in the low density stand on the sunny day, a 33% reduction in the needle complement of a vegetative tree resulted in a 17% reduction in the amount of CO_2 assimilated per day and the male cone-bearing tree assimilated 6% less CO_2 than the wholly vegetative tree. The same trends were shown on the moderately sunny day (23/9/87).

However, all three tree types in both stocking densities respired more CO_2 than they assimilated on the very cloudy day (21/9/87), i.e. they had a negative CO_2 balance over 24 hours. The vegetative tree with full needle complement had the largest negative balance because it had the highest dark respiration rate (Figure 7.4).

7.5.3 DISCUSSION

As a result of the larger needle complement the wholly vegetative trees absorbed more PAR compared to either vegetative trees or male cone-bearing trees with a 33% reduction in needle complement. However, in the lower tree crown the difference in the absorbed PAR was less pronounced, because the reduction in needle complement resulted in less shading and a higher transmittance of solar radiation through the tree crown and that of the surrounding trees.

It was expected that the needle reduction associated with the production of male cones would result in trees producing less photosynthate. A 33% reduction in needle complement resulted in a 10% and 15% reduction in assimilation in the high and low density stands, respectively. However, the older needles on the male cone-bearing branches have an increased quantum flux efficiency. MAESTRO predicted that on a bright and a moderately sunny day in September, male cone-bearing trees assimilated the same amount of CO_2 as wholly vegetative trees in the high density stand, and only slightly less (6%) in the low density stand. This small reduction in daily assimilation in the low density stand is the result of the slightly increased PAR absorbed by the needles within the crown of the wholly vegetative target tree because of self shading.

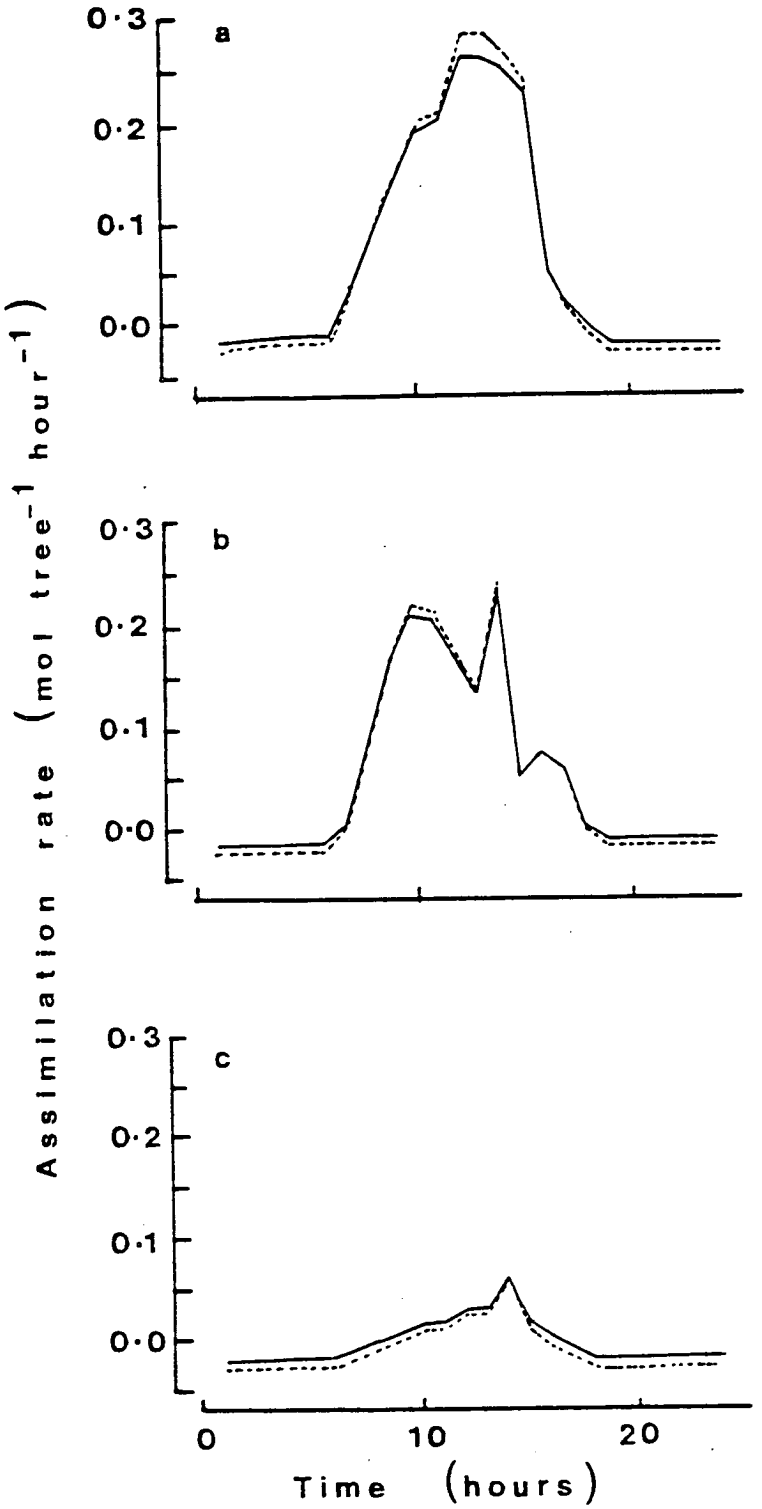


Figure 7.4 The diurnal course of assimilation on a a) sunny, b) moderately sunny and c) cloudy day in September, estimated by MAESTRO for a vegetative (....) and male cone-bearing (——) P. contorta tree within a forest stand.

The reduction in needle complement associated with male cone bearing was beneficial in terms of net CO_2 gain on cloudy days of very low quantum flux density in both stand densities. This was the direct result of the lower respiration of male cone-bearing trees because of the smaller needle complement. A previous study (Chapter 3) showed that needles on male cone-bearing branches do not have a significantly different rate of respiration compared with equivalent needles on vegetative branches.

Needles on the current year's growth of conifers are net importers of assimilates in the spring and early summer (Ericsson 1980). The reduction in needle complement associated with male cones is therefore, probably beneficial in terms of carbon requirement during the early growth of the shoots because male cone-bearing shoots would import less assimilates. This study has shown that in the autumn the higher quantum efficiency of the remaining needles on the tree compensates to a large extent for the reduction in needle complement.

In conclusion it is predicted that the reduction in needle complement associated with male cones will have only a very slight negative effect on the CO_2 assimilation of a tree.

7.6 SUMMARY

1) Generally the proportion of CO_2 efflux by female cones to that assimilated by the target tree was less than 3.0%. However in the high density stand on a very cloudy overcast day in August, the models estimated that the net respiration loss of a crop of 20 female cones was equivalent to 17.2% of the CO_2 assimilated by the target tree.

2) It was concluded that the net respiration loss of female cones probably does not represent a significant proportion of the CO_2 assimilated by a tree under most conditions. However on very cloudy overcast days the CO_2 lost as a result of respiration by female cones may make a significant impact on tree growth.

3) It was estimated that on a sunny day in autumn, the daily CO_2 assimilation of male cone-bearing trees in a high density stand was similar to that of vegetative trees, despite a 33% reduction in their needle complement. However in a low density stand of trees male cone-bearing trees would have a slightly reduced assimilation (6%) because of less shading.

4) It was estimated that a male cone-bearing tree had a lower respiration cost than a wholly vegetative tree by virtue of its reduced needle complement. The reduced respiration cost is particularly beneficial on cloudy days.

5) It was concluded that male cones only slightly reduce the assimilation of the trees which bore them.

CHAPTER 8

GENERAL SUMMARY AND CONCLUSIONS

8.1 SUMMARY

The aim of this study was to determine the influence of sexual structures on the growth of Pinus contorta (Dougl.) trees. It is important to know if male and female cone formation reduces the growth of trees, because seed production techniques currently used will probably result in an increased level of cone production in the forests of the future. It was clear from a review of the literature that some studies had found a detrimental influence of male and female cones on the growth of conifer trees, whereas others had found that the presence of sexual structures was associated with increased growth rates.

The research objectives of this study were to elucidate the differences in the inputs, outputs and allocation of carbon between reproductive and vegetative branches in both potted plants and field-grown trees (Figure 8.1). This was achieved by:

(i) measuring the CO_2 exchange rate of needles associated with female and male cones and determining the allocation of dry weight within a branch when the cones were fully developed (Chapter 2-4),

(ii) measuring and developing a mathematical model of the CO_2 exchange rate of female cones from a few weeks after pollination until they senesced. The CO_2 exchange rate of male cone-bearing buds could only be measured in the later stages of development because their CO_2 efflux was too small to be measured with existing equipment and there was insufficient time to build an appropriate leaf chamber (Chapter 5),

(iii) a potted plant system was successfully developed which would allow the inputs, outputs and allocation of carbon within a discrete system to be measured (Chapter 6). Unfortunately because of environmental and pathological problems insufficient potted

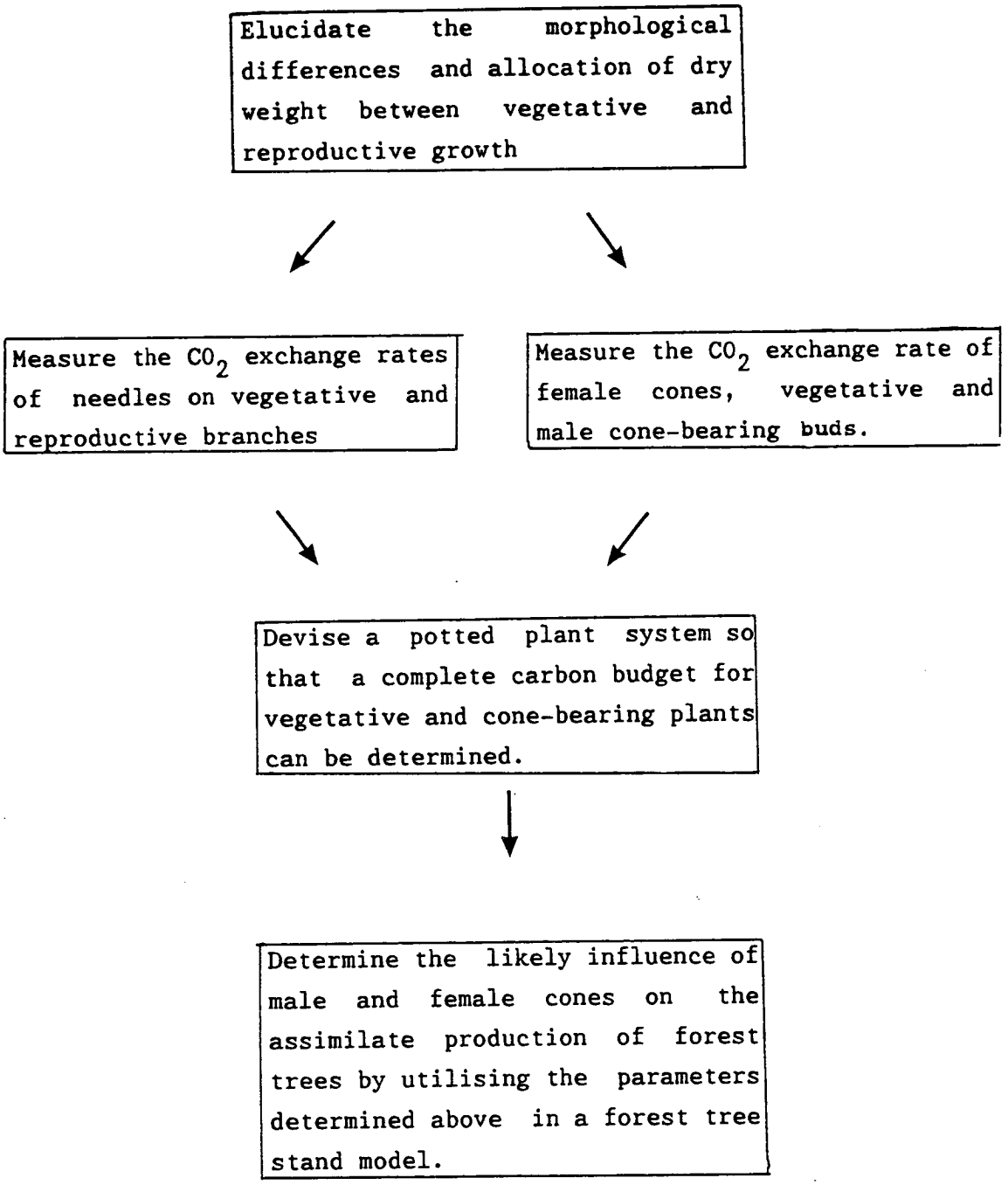


Figure 8.1 Flow diagram showing the lines of investigation followed in this project to determine the influence of reproductive structures on the morphology and physiology of P. contorta trees.

plants were obtained in this study to characterise the carbon economy of reproductive potted plants.

(iv) the CO_2 exchange rate of female and male cone-bearing trees within a forest stand were successfully estimated by using a simulation model (Chapter 8).

The results of these studies are summaries below.

8.1.1 MALE CONES

The investigation of male cones on the morphology and dry weight allocation of branches showed that compared with equivalent vegetative shoots, male cones reduced the photosynthetic potential of the shoots which bore them, by reducing the number of needles on the branch (Figure 8.2). It was estimated that over the whole male cone-bearing region of the tree crown 27% to 50% of the needles may be lost. In addition branches bearing male cones had significantly fewer lateral shoots. However in the spring just before the male cones shed their pollen, reproductive branch units had approximately 40% more dry weight than equivalent vegetative branches. The total dry weight of the needles on the parent shoot of both vegetative and male cone-bearing branch units was significantly associated with the number of needles and male cones on the current year's growth. It was suggested that both needle and male cones primordia developing within the winter buds influenced assimilate production and storage within the branch units, resulting in carbon reserves in the spring proportional to the number of needles and male cones. The influence of male cones on the assimilation rate of associated needles was therefore measured (Chapter 3 & 4).

There was no significant difference in the CO_2 exchange rate of needles on the parent shoots of male cone-bearing and vegetative branches in the spring or summer. However, in the autumn, the assimilation rate / quantum flux density response curve of needles on the parent shoot of male cone-bearing branches differed significantly from the response curve of needles on the parent shoot of vegetative branches, both in field-grown trees and on potted

Early spring
March/April

Male cone section of the bud accounts for ∝ 82% of the total dry weight (∝ 0.57 g).

70% refixation capacity, and an average dark respiration rate at 15°C of 0.009 μmol g^{-1} s^{-1}.

Late spring
May/June

Total current year's growth (∝ 1.12 g dry weight):

41% vegetative terminal

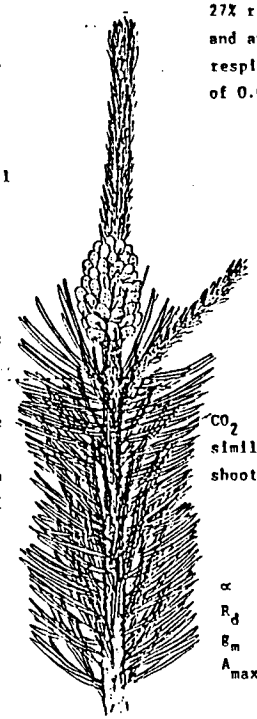
54% male cones

5% lateral shoots

Dry weight per needle significantly associated (+) with total dry weight of current year's growth.

27% refixation capacity and an average dark respiration rate at 15°C of 0.006 μmol g^{-1} s^{-1}.

CO_2 exchange rate similar to vegetative shoots.



	Male	Vegetative
∝	0.033	0.027
R_d	1.00	1.00
g_m	28.52	26.68
A_{max}	4.93	4.93

Autumn
October

Compared to equivalent vegetative shoots male cone bearing branches had 33% less needles and significantly less lateral shoots

The CO_2 exchange rate between male and vegetative shoots was not significantly different, but further study is need to confirm this result.

Male cone-bearing branches have significantly increased photosynthetic efficiancy compared with vegetative shoots.

	Male	Vegetative
∝	0.071	0.060
R_d	0.69	0.60
g_m	61.29	64.73
A_{max}	10.05	9.98

Figure 8.2 Summary of the influence of male cones on the morphology and physiology on P. contorta branches, (∝ = initial slope of photosynthetic quantum response curve (mol mol^{-1}), R_m = mesophyll conductance (mmol m^{-2} s^{-1}), R_d = dark respiration rate (μmol m^{-2} s^{-1}), A_{max} = maximum assimilation rate (μmol m^{-2} s^{-1}) at quantum flux density's > 1500 μmol m^{-2} s^{-1}).

plants (Figure 8.2). Needles on the parent shoot of male cone-bearing branches had a higher quantum use efficiency and a higher maximum assimilation rate. It was suggested that plant growth regulators originating in the developing bud may control the photosynthetic rate of needles on the parent shoot. Unfortunately, this hypothesis was not tested experimentally because of insufficient material and time.

Needle and male cone primordia contain chloroplasts and are, therefore, capable of contributing to their own carbon economy. The extent to which this occurs was measured and is reported in Chapter 5 (Figure 8.2). In the spring 6-8 weeks before the male cones shed their pollen, male cone-bearing buds had almost twice the dark respiration rate compared with vegetative buds. Although male cone primordia refixed CO_2 as efficiently as needle primordia, the respiration cost of male cone-bearing buds was higher than equivalent vegetative buds. Two to three weeks prior to pollen shed the refixation capacity of male cones was reduced presumably as a result of maturation processes. The dark respiration rate of male cone-bearing buds was also reduced at this time and was similar to vegetative buds. Male cone-bearing buds were therefore considered to reduce significantly the efflux of CO_2 by refixation but were considered a larger carbon drain on the tree during early spring than vegetative buds.

The influence of the reduction in needle complement and increase in quantum efficiency of needles associated with male cones was estimated by using the simulation model MAESTRO (Chapter 7). This model has previously been validated in stands of Picea sitchensis and Pinus radiata and the photosynthesis submodel was used to determine the quantum efficiency of needles on vegetative and male cone-bearing branches in Chapter 3 and 4. MAESTRO was used to estimate the daily assimilation of a target tree within a forest stand. It was estimated that the reduction in needle complement on male cone-bearing trees in the autumn only slightly reduced the assimilation of the trees which bore them on sunny days because of the increase in photosynthetic efficiency of the older needles and

reduced self-shading, particularly in the lower crown. Whereas on cloudy days the reduction in needle complement was beneficial to the carbon economy of the tree, because fewer needles were respiring. Similarly, it was suggested that early in the growing season the reduction in needle number would be beneficial to the carbon economy of the tree because the current year's needles are net importers of assimilates until they are approximately 33% - 50% expanded.

These studies have shown, therefore, that male cones significantly alter the morphology and physiology of the shoots which bear them. The reduction in needle complement is, however, compensated for by both increased quantum efficiency of surrounding needles and increased transmittance of light into the lower crown of male cone-bearing trees. It was concluded, therefore, that the production of male cones is probably not detrimental to vegetative growth of the trees which bear them.

8.1.2 FEMALE CONES

Studies on branches from field-grown trees (Chapter 2) showed that female cones do not replace lateral shoots, although they do reduce the number of latent buds (Figure 8.3). Latent buds were considered as an 'insurance strategy' such that if a branch was broken a latent bud would differentiate needles and grow. It was estimated that a cone crop of four female cones would reduce the number of potential lateral buds on a branch unit by only 29%, but would not reduce the number which differentiated and grew the following spring. It was considered, therefore, that female cones would not significantly reduce the number of lateral branches in normal growing conditions.

The presence of two year-old (n2) female cones did not reduce the length of the terminal or lateral shoots at the end of the growing season. In fact, female cones were associated with increased lateral shoot growth. Branch units bearing n2 female cones also had more dry weight then equivalent vegetative branches, but the dry weight of the terminal and lateral shoots was not reduced. The increase in dry weight of female cone-bearing branches may have resulted from an increased photosynthetic efficiency similar to that

Year 1
Autumn/winter
September/January

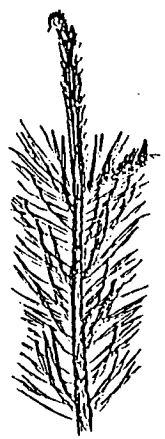
Female cone (≃ 0.75 g) account for ≃ 1% of the total dry veight in the autumn (≃ 27 g).

Female cone has: 95% refixation capacity, and an average dark respiration rate at 15°C of 0.00275 μmol g^{-1} s^{-1}.

Year 2
Late spring
May/June

Female cones do not replace groving lateral shoots.

When barkringed at the base of the parent shoot the dry veight of the female cones is reduced by ≃ 25%.



Female cone has: 80% refixation capacity and an average dark respiration rate at 15°C of 0.0070 μmol g^{-1} s^{-1}.

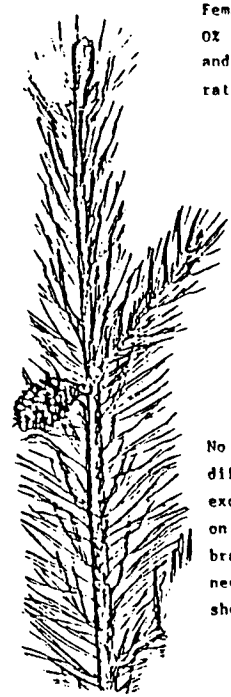
Needles on the parent have significantly higher respiration rate but not significantly different photosynthetic rate compared with similar vegetative shoots.-

	Female	Vegetative
α	0.029	0.027
R_d	1.29	1.00
g_m	29.04	26.68
A_{max}	5.71	4.93

Year 2
Autumn
October

Length of terminal and lateral shoots not reduced on female cone-bearing branches. Female cones (≃ 4.5 g each) not significantly associated with total dry veight of the current year's vegetative grovth (≃ 35.7 g). Female cones 20% terminal shoot 41% lateral shoots 39% of the total dry veight.

Dry veight per needle significantly associated (+) with total dry veight of current year's grovth.



Female cone has: 0% refixation capacity and zero respiration rate of female cone.

No significant difference in the CO_2 exchange rate of needles on female cone-bearing branches compared with needles on vegetative shoots

	Female	Vegetative
α	0.073	0.060
R_d	0.76	0.60
g_m	61.31	64.73
A_{max}	9.37	9.98

Figure 8.3 Summary of the influence of female cones on the morphology and physiology on P. contorta branches, (α = initial slope of photosynthetic quantum response curve (mol mol^{-1}), g_m = mesophyll conductance (mmol m^{-2} s^{-1}), R_d = dark respiration rate (μmol m^{-2} s^{-1}), A_{max} = maximum assimilation rate (μmol m^{-2} s^{-1}) at quantum flux density's > 1500 μmol m^{-2} s^{-1}).

found on male cone-bearing branches, but this was not established with the same degree of certainty.

The assimilation rate / quantum flux density response curve of needles on the parent shoot of female cone-bearing branches did not differ significantly from needles on the parent shoot of closely associated vegetative branches (Chapter 3). It was suggested that female cones may produce plant growth regulators which influence the assimilation rate of both the needles on the branches which bear them and the needles on closely associated branches. No difference, therefore, would be measurable in this study. Unfortunately it was not possible to measure the CO_2 exchange of needles associated with female cones independently, because there was insufficient suitable material.

The pattern of cone growth and the rate of dark respiration throughout the life cycle of the female cones was found to be consistent with current theories of growth and maintenance respiration. The dark respiration rate declined through the winter as the temperature fell, presumably as a result of reduced enzyme and protein turnover (i.e. maintenance respiration). The phase of maximum dark respiration rate in the spring preceded the period of maximum cone growth by a few weeks which was probably the result of a period of high metabolic activity before the cone measurably increased in size. The phase of declining dark respiration rates in the autumn correlated with maturation of the cone.

Female cones were found to be capable of significant refixation until a few months before shedding seed. The model developed in Chapter 5 estimated that throughout the year female cone refixation reduced the efflux of CO_2 by 25%. It was also estimated that female cones respired 31% of their final weight in carbon. The contribution of female cones to their own carbon economy was found to be primarily dependent on the temperature and quantum flux density. Consequently, annual variation in the weather may significantly influence the effect of female cones on tree growth.

The proportion of CO_2 lost as a result of respiration by female cones to that assimilated by the needles on a tree was estimated from mathematical models. MAESTRO was used to estimate the daily CO_2 exchange of needles on a target tree within a stand and the model developed in this study (Chapter 5) was used to estimate the net daily respiration loss of a female cone crop. It was shown that the female cone crop generally respired only a very small percentage (< 3%) of the CO_2 assimilated by the tree. However on a cloudy day in August a crop of 20 cones would respire as much as 17% of the CO_2 assimilated by the tree. These studies indicate the importance of environmental variables in determining the influence of reproductive structure on the growth of trees. Because reproductive structures are an important sink for assimilates (see section 1.5.4), cones may result in reduced growth in years with dull weather.

These studies have shown that female cones alter the morphology of the branches which bear them by reducing the number of latent buds. However this study did not detect differences in physiology between female cone-bearing branches and closely associated vegetative branches. It was concluded that the production of female cones was not detrimental to the vegetative growth of the tree which bore them.

8.2 FUTURE STUDIES

It is very difficult to determine the influence of reproductive structures independently from other associated structures. Similarly local environmental variables and genetic variation further complicate the investigation of the carbon economy of reproductive trees. These factors have severly limited this study. It was planned to overcome these problems by constructing a complete carbon budget for reproductive and vegetative potted plants, and to model the growth of both cones and vegetative parts by measuring the uptake and release of CO_2 from these plants (Chapter 6). A reproductive cuttings system was successfully established in this study. Shoots bearing female and male cones were successfully rooted and established on a suitable medium for CO_2 exchange studies and dry weight partitioning studies of the roots and shoots.

Unfortunately insufficient cuttings were propagated during this study primarily, it is believed, because of environmental and pathological rather than physiological factors. This approach is considered important and justifies further study.

Investigating the CO_2 exchange of vegetative and reproductive potted plants, while concurrently assessing the allocation of assimilates and the growth of the shoots, stem, roots and cones would enhance our understanding of the principles governing assimilate allocation. As the most important advances in the productivity of commercial crops have been achieved by altering the allocation of assimilates to the harvestable product (Pulkkinen et al. 1989), such studies should supply fundamental information of value to the tree breeders and foresters. The system outlined in Chapter 6 would also allow the supply and uptake of nutrients to be investigated. Further studies into the possible effects of elevated CO_2 and other pollutant gases which may enhance cone crops, by virtue of their effect as stress treatments, would also be possible on such a defined system in controlled conditions.

The reproductive potted plant system would also allow investigation of the mechanisms by which reproductive structures influence assimilation to be determined. Reproductive structures have been associated with increased assimilation in many plants but the mechanisms by which this occurs are poorly understood. Because male cone buds can have between 1 and 100 male cones a system with variable cone load (i.e. sink strength) could be obtained and by limiting the number of needles per cutting the source strength could also be manipulated. The reproductive cutting system would, therefore, allow experimentation into the interactions between source and sink strengths of plants in a discrete system where all the inputs and outputs could be determined.

It is apparent when studying coniferous trees that male and female cones are formed on fairly strict zones within the architecture of the trees. Female cones are found on vigorous branches high in the tree crown, while male cones are located on smaller branches lower in the tree crown. Studies have shown an interaction between the

length of a branch, it's position within the tree crown and the sex of cones which develop on a branch (see Dick et al. in prep.) It would appear that such a system could be elucidated by a modelling approach and determining the mathematical functions for these relationships. This may help to identify the processes of sex determination in conifers which could lead to significantly increased seed yields in commercial orchards.

8.3 CONCLUDING REMARKS

The findings reported here are clearly at variance with the basic assumption made by many workers in the field, quoted by Linder & Rook (1984):

> "The basic assumption in C (carbon) balance studies is that photosynthetic production is limited and if a plant uses more energy in one way less C will be available to meet other demands and growth of other parts. It is contended therefore that flowering and seed production largely takes place at the expense of vegetative growth."

This project has shown that needles on the parent shoot of male cone-bearing branches have an increased photosynthetic rate and that both male and female cones significantly contribute to their own carbon economy by refixing CO_2 when illuminated. Rather than the static system envisaged by many workers in this field, it would appear from these studies that mechanisms operate within trees to ensure that vegetative growth does not suffer as a result of cone production in normal growing conditions. In evolutionary terms it would be advantageous for a tree to grow quickly and produce cones while continuing to grow and compete effectively with its neighbours. It is suggested, therefore, that reproductive structures are not often a significant drain on the carbon reserves of trees. Rather trees have, through natural selection, developed mechanisms to ensure maximum vegetative and reproductive growth simultaneously.

One such mechanism may be the accumulation of reserves prior to shoot and cone growth. The branch studies showed that both vegetative and male cone-bearing branches in the spring apparently had carbohydrates reserves (infered from dry weight data) in

proportion to the number of needles and male cones enclosed within the winter bud. This finding implies that the needle and male cone primordia in the overwintering bud influenced the process of dry weight accumulation in the autumn and spring. Feed-back mechanisms of this sort may, therefore, control the following year's growth. The mechanism by which this was achieved was not investigated but it would appear resonable to hypotheses that plant growth regulators arising in the developing bud affected the photosynthetic activity of the branch such that it accumulated reserves for the future development of the following year's growth (see reviews Bidwell 1983, Weaver & Johnson 1985). The influence of male cone-bearing buds on the CO_2 exchange rate of the needles immediately adjacent to them was not rigorously investigated, but the data presented here are not at variance with the idea that the photosynthetic rate is strongly influenced by the developing bud. Female cones may have a similar influence on the surrounding needles although only a trend was detected in this project. This was possibly because the vegetative branches used as a comparison were also influenced by the presence of female cone.

The presence of chlorophyll in cones (Kozubov et al. 1985) and the reduction of CO_2 efflux when the cones are illuminated is another mechanism which ensures cones are not produced at the expense of vegetative growth. The location of male and female cones within different parts of the tree crown may also be considered important in this respect. Female cones are commonly found on the vigorous branches in the distal regions of the crown were they are subjected to high quantum flux densitys and therefore well situated to refix CO_2. Male cones, on the other hand, are commonly found in the lower proximal regions of the tree crown, but because their maximum refixation capacity occurs during the dull winter months when radiation is largely diffuse, this lower position in the tree crown is probably not important. Male cones also reduce the needle complement of the branches which bear them. However because they are located in the lower proximal regions of the tree crown where attenuation of light is strong, the reduction in needle complement is not particularly detrimental to assimilation by the crown as a whole because of increased light penetration to the lower canopy.

These studies suggest that breeding from floriferous individuals will not be detrimental to the growth of future forests in normal conditions and may have significant ecological advantages because cones are an important food source for many animals, e.g. Sciurus spp. (squirrels), Loxia spp. (crossbills) and Carduelis spp. (siskins) (see Staines et al. 1987).

Further work is needed to completely clarify the influence of male and female cones on the carbon economy of the trees which bears them. However, it is clear from the studies reported here that it is no longer acceptable to consider that cones result in an reduction in stem weight equivalent to their own weight. Trees appear to be adapted to produce simultaneously strong vegetative and reproductive growth in natural growing conditions.

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APPENDIX 1 - LIST OF SYMBOLS

A	gross assimilation rate (μmol m^{-2} s^{-1})
A_{max}	the asymptote of the quantum response curve (μmol m^{-2} s^{-1})
C_a	ambient concentration of CO_2 (μmol mol^{-1})
C_i	intercellular concentration of CO_2 (μmol mol^{-1})
D_a	ambient vapour pressure (kPa)
D_l	water vapour pressure difference across the leaf surface (kPa)
g_s	stomatal conductance (mmol m^{-2} s^{-1})
g_m	mesophyll conductance (mmol m^{-2} s^{-1})
Q	quantum flux density (μmol m^{-2} s^{-1})
R_d	dark respiration (μmol m^{-2} s^{-1})
α	the initial slope of the quantum response curve
β	quantum flux coefficient
Γ	CO_2 compensation concentration point (μmol mol^{-1})
η	temperature coefficient
θ	convexity coefficient i.e. the degree of bending between α and A_{max} in a quantum response curve
ω	refixation capacity
ς	ratio of the wavelengths absorbed by phytochrome